Mendel's Law of Independent Assortment

Mendel performed a second series of crosses in which true-breeding plants differed in two traits. For example, he crossed tall plants having green pods with short plants having yellow pods (Fig. 11.5). The F_1 plants showed both dominant characteristics. As before, Mendel then allowed the F_1 plants to self-pollinate. This F_1 cross is known as a **dihybrid cross** because the plants are hybrid in two ways. Two possible results could occur in the F_2 generation:

1. If the dominant factors (*TG*) always segregate into the F_1 gametes together, and the recessive factors (*tg*) always stay together, then there would be two phenotypes among the F_2 plants—tall plants with green pods and short plants with yellow pods.
2. If the four factors segregate into the F_1 gametes independently, then there would be four phenotypes among the F_2 plants—tall plants with green pods, tall plants with yellow pods, short plants with green pods, and short plants with yellow pods.

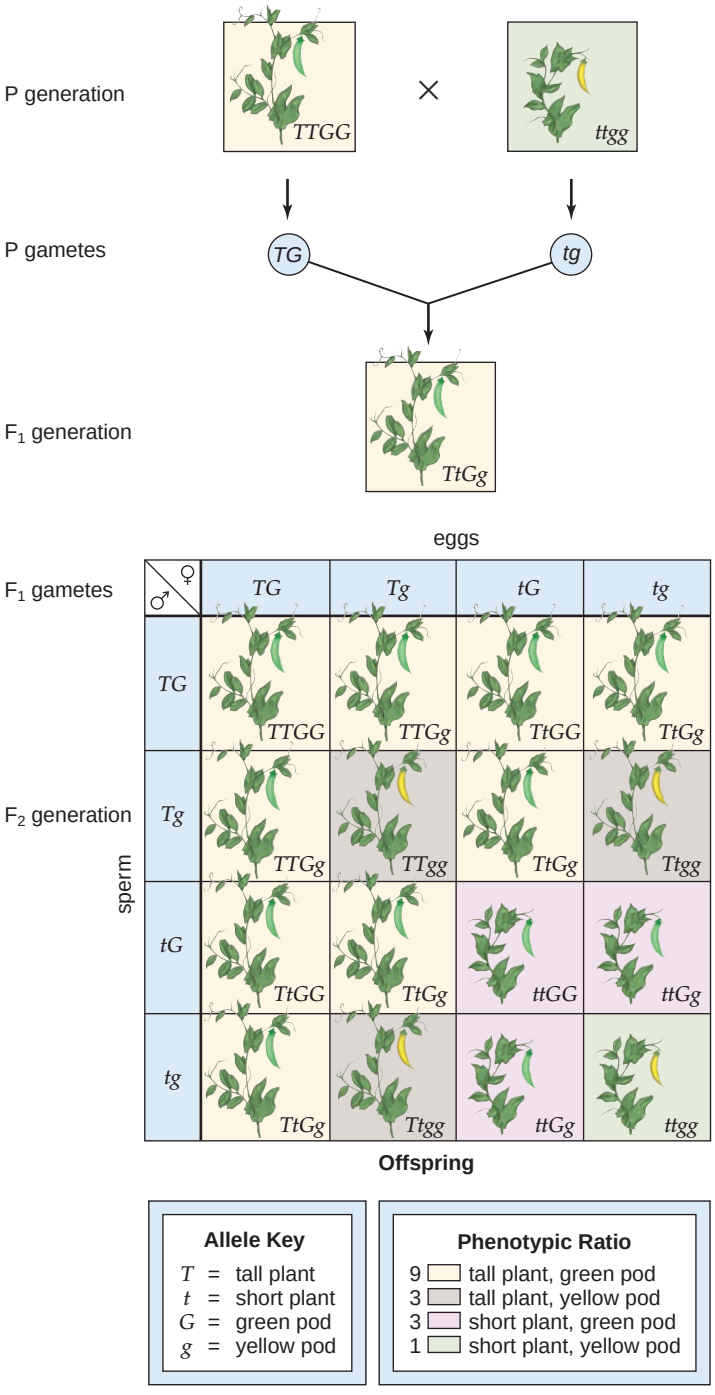


FIGURE 11.5 Dihybrid cross done by Mendel.

P generation plants differ in two regards—length of the stem and color of the pod. The F_1 generation shows only the dominant traits, but all possible phenotypes appear among the F_2 generation. The 9:3:3:1 ratio allowed Mendel to deduce that factors segregate into gametes independently of other factors.

Figure 11.5 shows that Mendel observed four phenotypes among the F_2 plants, supporting the second hypothesis. Therefore, Mendel formulated his second law of heredity—the law of independent assortment.

The law of independent assortment states the following:

- Each pair of factors segregates (assorts) independently of the other pairs.
- All possible combinations of factors can occur in the gametes.

The law of independent assortment applies only to alleles on different chromosomes. Each chromosome carries a large number of alleles.

Again, we know that the process of meiosis explains why the F_1 plants produced every possible type of gamete and, therefore, four phenotypes appear among the F_2 generation of plants. As was explained in the Science Focus on page 195, there are no rules regarding the alignment of homologues at the metaphase plate—the daughter cells produced have all possible combinations of alleles. The possible gametes are the two dominants (such as *TG*), the two recessives (such as *tg*), and the ones that have a dominant and recessive (such as *Tg* and *tG*). When all possible sperm have an opportunity to fertilize all possible eggs, *the expected phenotypic ratio of a dihybrid cross is always 9:3:3:1.*

Check Your Progress 11.2B

1. In fruit flies, *L* = long wings and *l* = short wings; *G* = gray body and *g* = black body. List all possible gametes for a heterozygote.
2. What phenotypic ratio is expected when two dihybrids reproduce?

science focus

Mendel's Laws and Meiosis

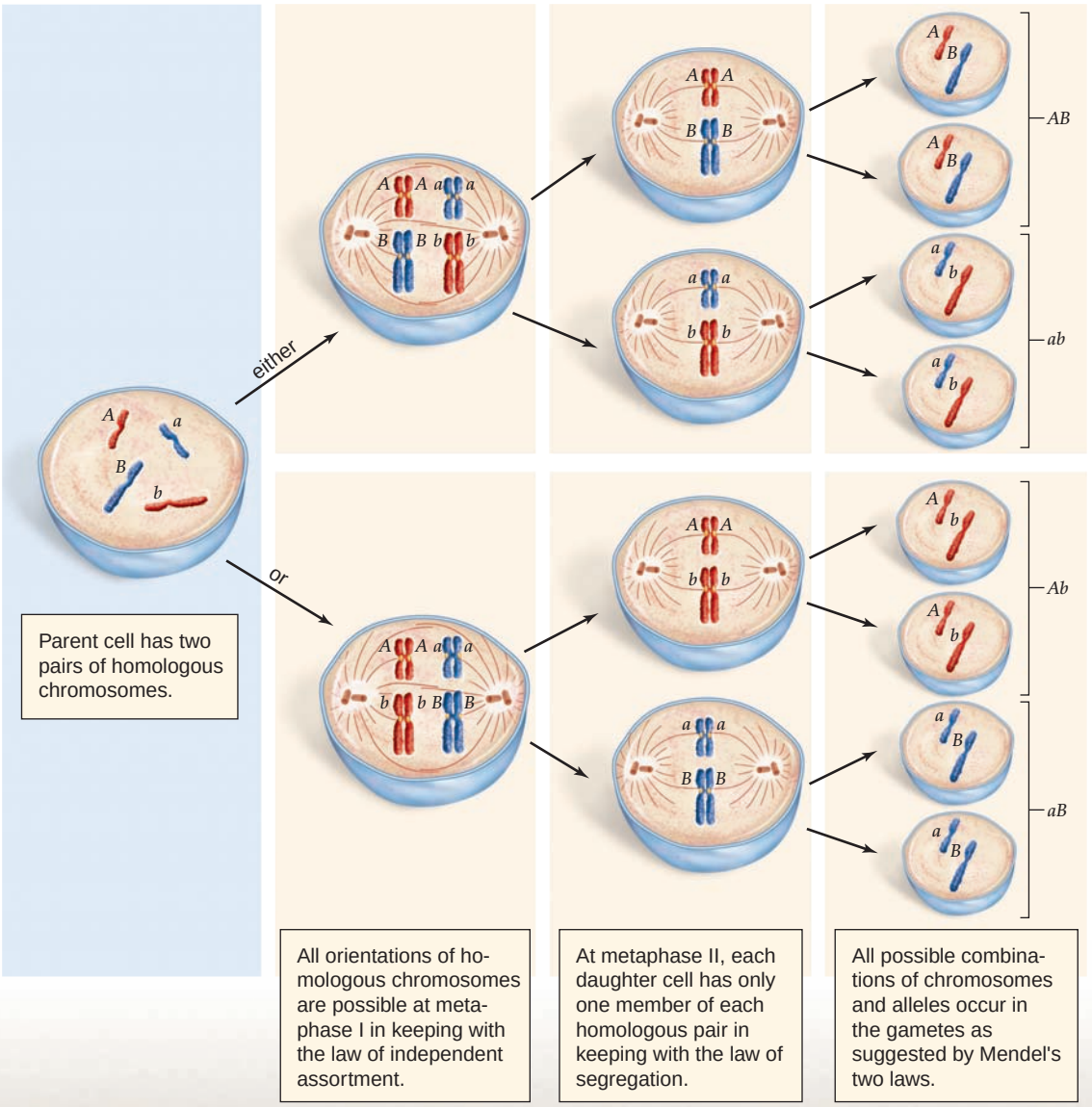
Today, we realize that the genes are on the chromosomes and that Mendel's laws hold because of the events of meiosis. Figure 11A assumes a parent cell that has two homologous pairs of chromosomes and that the alleles *A*,*a* are on one pair and the alleles *B*,*b* are on the other pair. Following duplication of the chromosomes, the parent cell undergoes meiosis as a first step toward the production of gametes. At metaphase I, the homologous pairs line up independently and, therefore, all alignments of homologous chromosomes can occur at the metaphase plate. Then the pairs of homologous chromosomes separate.

In keeping with Mendel's law of independent assortment and law of segregation, each pair of chromosomes and alleles segregates independently of the other pairs. It matters not which member of a homologous pair faces which spindle pole. Therefore, the daughter cells from meiosis I have all possible combinations of alleles. One daughter cell has both dominant alleles, namely *A* and *B*. Another daughter cell has both recessive alleles, namely *a* and *b*. The other two are mixed: *A* with *b* and *a* with *B*. Therefore, all possible combinations of alleles occur in the gametes.

When you form the gametes for any genetic cross, you are following the dictates of Mendel's laws but also mentally taking the chromosomes and alleles through the process of meiosis. We can also note that fertilization restores both the diploid chromosome number and the paired condition of alleles in the zygote.

FIGURE 11A Independent assortment and segregation during meiosis.

Mendel's laws hold because of the events of meiosis. The homologous pairs of chromosomes line up randomly at the metaphase plate during meiosis I. Therefore, the homologous chromosomes, and alleles they carry, segregate independently during gamete formation. All possible combinations of chromosomes and alleles occur in the gametes.



Mendel's Laws of Probability

The diagram we have been using to calculate the results of a cross is called a Punnett square. The **Punnett square** allows us to easily calculate the chances, or the probability, of genotypes and phenotypes among the offspring. Like flipping a coin, an offspring of the cross illustrated in the Punnett square in Figure 11.6 has a 50% (or ½) chance of receiving an *E* for unattached earlobe or an *e* for attached earlobe from each parent:

The chance of *E* = ½
The chance of *e* = ½

How likely is it that an offspring will inherit a specific set of two alleles, one from each parent? The *product rule* of probability tells us that we have to multiply the chances of independent events to get the answer:

1. The chance of *EE* = ½ × ½ = ¼
2. The chance of *Ee* = ½ × ½ = ¼
3. The chance of *eE* = ½ × ½ = ¼
4. The chance of *ee* = ½ × ½ = ¼

The Punnett square does this for us because we can easily see that each of these is ¼ of the total number of squares. How do we get the phenotypic results? The *sum rule* of probability tells us that when the same event can occur in more than one way, we can add the results. Because 1, 2, and 3 all result in unattached earlobes, we add them up to know that the chance of unattached earlobes is ¾, or 75%. The chance of attached earlobes is ¼, or 25%. The Punnett square doesn't do this for us—we have to add the results ourselves.

Another useful concept is the statement that "chance has no memory." This concept helps us know that each child has the same chances. So, if a couple has four children, each child has a 25% chance of having attached earlobes. This may not be significant if we are considering earlobes. It does become significant, however, if we are considering a recessive genetic disorder, such as cystic fibrosis, a debilitating respiratory illness. If a heterozygous couple has four children, each child has a 25% chance of inheriting two recessive alleles, and all four children could have cystic fibrosis.

We can use the product rule and the sum rule of probability to predict the results of a dihybrid cross, such as the one shown in Figure 11.5. The Punnett square carries out the multiplication for us, and we add the results to find that the phenotypic ratio is 9:3:3:1. We expect these same results for each and every dihybrid cross. Therefore, it is not necessary to do a Punnett square over and over again for either a monohybrid or a dihybrid cross. Instead, we can simply remember the probable results of 3:1 and 9:3:3:1. But we have to remember that the 9 represents the two dominant phenotypes together, the 3's are a dominant phenotype with a recessive, and the 1 stands for the two recessive phenotypes together. This tells you the probable phenotypic ratio among the offspring, but not the chances for each possible phenotype. Because the Punnett square has 16 squares, the chances are 9/16 for the two dominants together, 3/16 for the dominants with each recessive, and 1/16 for the two recessives together.

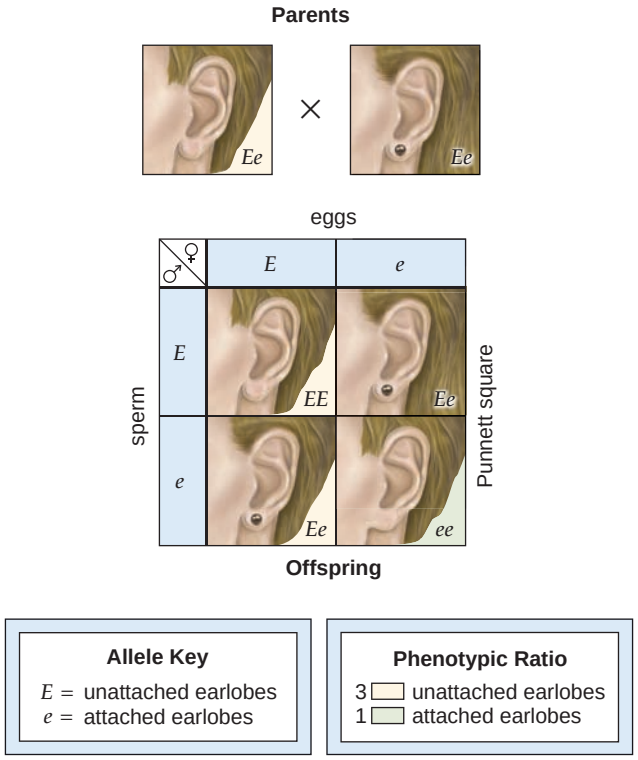


FIGURE 11.6 Punnett square.

Use of Punnett square to calculate probable results in this case a 3 : 1 phenotypic ratio.

Mendel counted the results of many similar crosses to get the probable results, and in the laboratory, we too have to count the results of many individual crosses to get the probable results for a monohybrid or a dihybrid cross. Why? Consider that each time you toss a coin, you have a 50% chance of getting heads or tails. If you tossed the coin only a couple of times, you might very well have heads or tails both times. However, if you toss the coin many times, you are more likely to finally achieve 50% heads and 50% tails.

Check Your Progress 11.2C

1. In pea plants, yellow seed color is dominant over green seed color. When two heterozygous plants are crossed, what percentage of plants would have yellow seeds? Green seeds?
2. In humans, having freckles (*F*) is dominant over having no freckles (*f*) . A man with freckles reproduces with a woman with freckles, but the children have no freckles. What chance did each child have for having freckles?
3. In humans, short fingers (*S*) are dominant over long fingers (*s*). Without doing a Punnett square, what phenotypic ratio is probable when a dihybrid for freckles and fingers reproduces with another having the same genotype? Describe these offspring. What are the chances of an offspring with no freckles and long fingers?

Testcrosses

To confirm that the F_1 plants of his one-trait crosses were heterozygous, Mendel crossed his F_1 generation plants with true-breeding, short (homozygous recessive) plants. Mendel performed these so-called **testcrosses** because they allowed him to support the law of segregation. For the cross in Figure 11.7, he reasoned that half the offspring should be tall and half should be short, producing a 1:1 phenotypic ratio. His results supported the hypothesis that alleles segregate when gametes are formed. In Figure 11.7*a*, the homozygous recessive parent can produce only one type of gamete—*t*—and so the Punnett square has only one column. The use of one column signifies that all the gametes carry a *t*. *The expected phenotypic ratio for this type of one-trait cross (heterozygous × recessive) is always 1:1.*

One-Trait Testcross

Today, a one-trait testcross is used to determine if an individual with the dominant phenotype is homozygous dominant (e.g., *TT*) or heterozygous (e.g., *Tt*). Since both of these genotypes produce the dominant phenotype, it is not possible to determine the genotype by observation. Figure 11.7*b* shows that if the individual is homozygous dominant, all the offspring will be tall. Each parent has only one type of gamete and, therefore, a Punnett square is not required to determine the results.

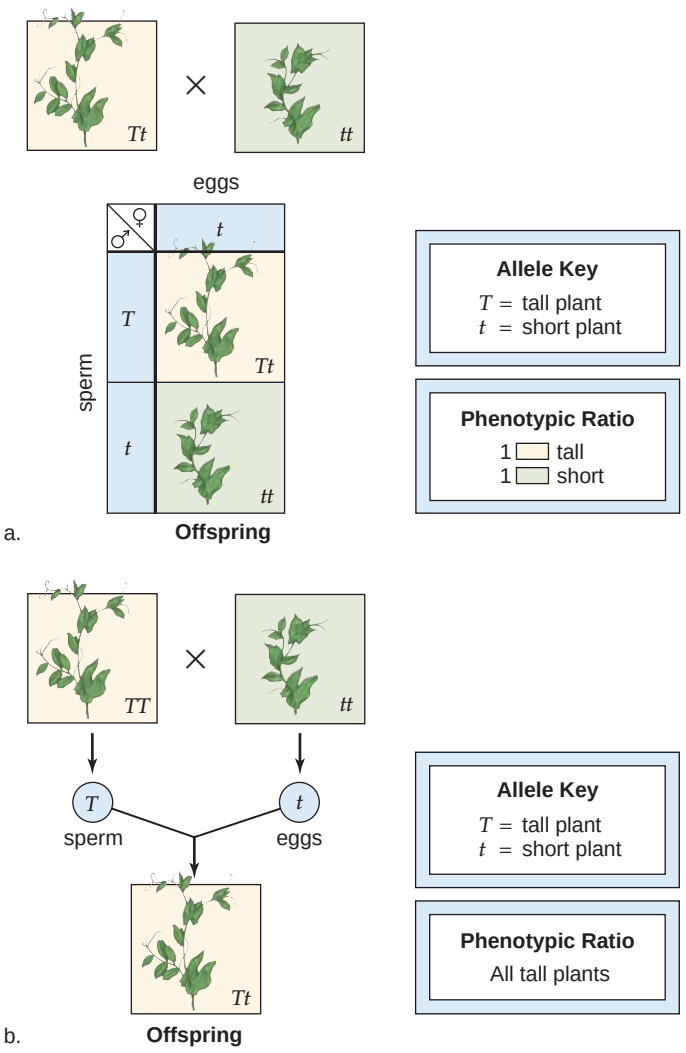


FIGURE 11.7 One-trait testcrosses.

a. One-trait testcross when the individual with the dominant phenotype is heterozygous. **b.** One-trait testcross when the individual with the dominant phenotype is homozygous.

Two-Trait Testcross

When doing a two-trait testcross, an individual with the dominant phenotype is crossed with one having the recessive phenotype. Suppose you are working with fruit flies in which:

L = long wings	*G* = gray bodies
l = vestigial (short) wings	*g* = black bodies

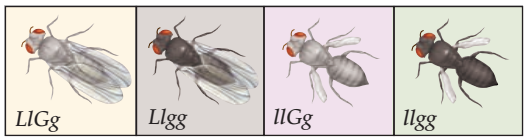
You wouldn't know by examination whether the fly on the left was homozygous or heterozygous for wing and body color. In order to find out the genotype of the test fly, you cross it with the one on the right. You know by examination that this vestigial-winged and black-bodied fly is homozygous recessive for both traits.

If the test fly is homozygous dominant for both traits with the genotype *LLGG*, it will form only one gamete: *LG*. Therefore, all the offspring from the proposed cross will have long wings and a gray body.

However, if the test fly is heterozygous for both traits with the genotype *LlGg*, it will form four different types of gametes:

Gametes: *LG* *Lg* *lG* *lg*

and could have four different offspring:



The presence of the offspring with vestigial wings and a black body shows that the test fly is heterozygous for both traits and has the genotype *LlGg*. Otherwise, it could not have this offspring. In general, you will want to remember that *the expected phenotypic ratio for this type of two-trait cross (heterozygous for two traits × recessive for both traits) is always 1:1:1:1.*

Check Your Progress 11.2D

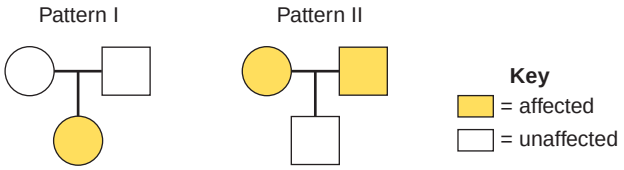
1. A heterozygous fruit fly (*LlGg*) is crossed with a homozygous recessive (*llgg*). What are the chances of offspring with long wings and a black body?
2. Using the key above for fruit flies, what are the most likely genotypes of the parents if a student gets the following phenotypic results? **a.** 1:1:1:1 **b.** 9:3:3:1
3. In horses, trotter (*T*) is dominant over pacer (*t*). A trotter is mated to a pacer, and the offspring is a pacer. Give the genotype of all the horses.

Mendel's Laws and Human Genetic Disorders

Many traits and disorders in humans, and other organisms also, are genetic in origin and follow Mendel's laws. These traits are controlled by a single pair of alleles on the autosomal chromosomes. An **autosome** is any chromosome other than a sex (X or Y) chromosome.

Autosomal Patterns of Inheritance

When a genetic disorder is autosomal dominant, the normal allele (*a*) is recessive, and an individual with the alleles *AA* or *Aa* has the disorder. When a genetic disorder is autosomal recessive, the normal allele (*A*) is dominant, and only individuals with the alleles *aa* have the disorder. A pedigree shows the pattern of inheritance for a particular condition and can be used by genetic counselors to determine whether a condition is dominant or recessive. Consider these two possible patterns of inheritance:



In both patterns, males are designated by squares and females by circles. Shaded circles and squares are affected individuals. The shaded boxes do not indicate whether the condition is dominant or recessive, only that the individual exhibits the trait. A line between a square and a circle represents a union. A vertical line going downward leads, in these patterns, to a single child. (If there are more children, they are placed off a horizontal line.) Which pattern of inheritance (I or II) do you suppose represents an autosomal dominant characteristic, and which represents an autosomal recessive characteristic?

In pattern I, the child is affected, but neither parent is; this can happen if the condition is recessive and both parents are *Aa*. Notice that the parents are **carriers** because they appear normal (do not express the trait) but are capable of having a child with the genetic disorder. In pattern II, the child is unaffected, but the parents are affected. This can happen if the condition is dominant and the parents are *Aa*.

Figure 11.8 shows other ways to recognize an autosomal recessive pattern of inheritance, and Figure 11.9 shows other ways to recognize an autosomal dominant pattern of inheritance. In these pedigrees, generations are indicated by Roman numerals placed on the left side. Notice in the third generation of Figure 11.8 that two closely related individuals have produced three children, two of which have the affected phenotype. In this case, a double line denotes consanguineous reproduction, or inbreeding, which is reproduction between two closely related individuals. This illustrates that inbreeding significantly increases the chances of children inheriting two copies of a potentially harmful recessive allele.

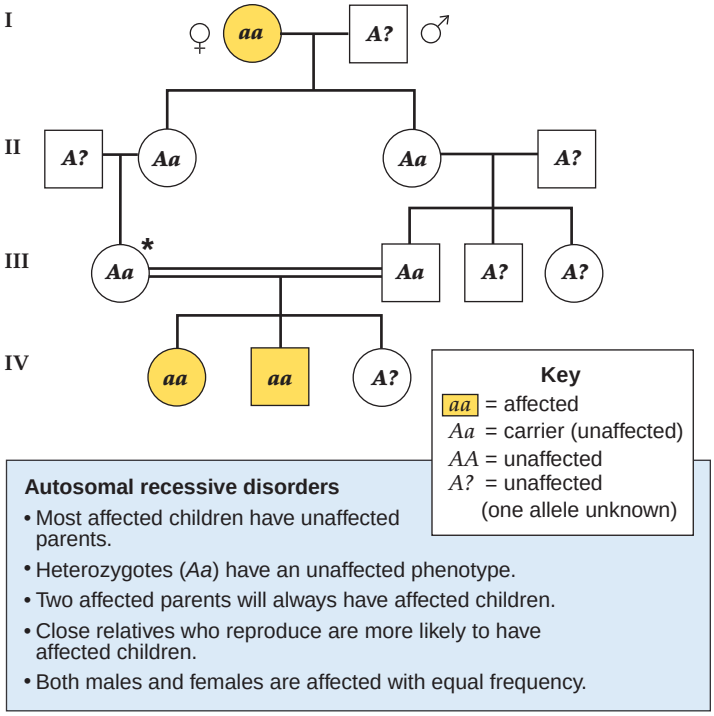


FIGURE 11.8 Autosomal recessive pedigree.

The list gives ways to recognize an autosomal recessive disorder. How would you know the individual at the asterisk is heterozygous?[1]

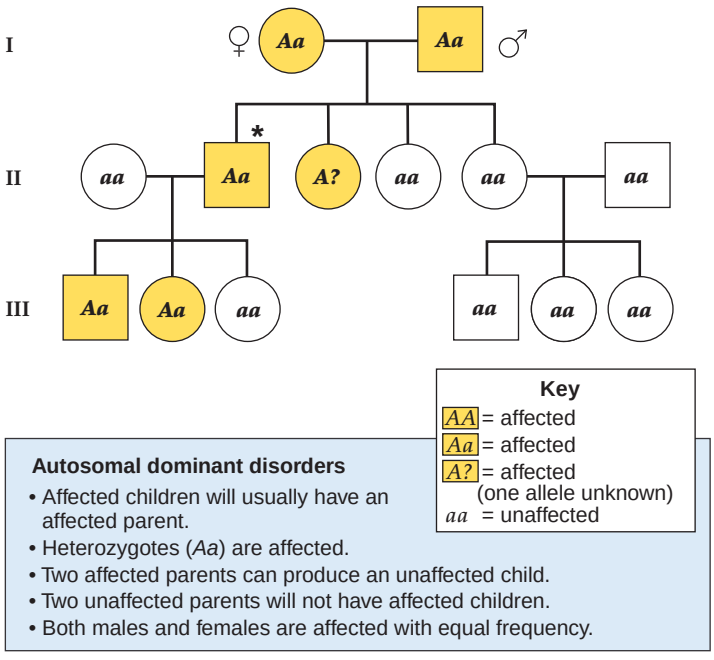


FIGURE 11.9 Autosomal dominant pedigree.

The list gives ways to recognize an autosomal dominant disorder. How would you know the individual at the asterisk is heterozygous?[1]

1. See Appendix A for answers.

Autosomal Recessive Disorders

In humans, a number of autosomal recessive disorders have been identified. Here, we discuss methemoglobinemia, cystic fibrosis, and Niemann-Pick disease.

Methemoglobinemia

Methemoglobinemia is a relatively harmless disorder that results from an accumulation of methemoglobin in the blood. While this disorder has been documented for centuries, the exact cause and genetic link remained mysterious. Although rarely mentioned, hemoglobin, the main oxygen-carrying protein in the blood, is usually converted at a slow rate to an alternate form called methemoglobin. Unlike hemoglobin, which is bright red when carrying oxygen, methemoglobin has a bluish color, similar to that of oxygen-poor blood. Although this process is harmless, individuals with methemoglobinemia are unable to clear the abnormal blue protein from their blood, causing their skin to appear bluish-purple in color (Fig. 11.10)!

A persistent and determined physician finally solved the age-old mystery of what causes methemoglobinemia by doing blood tests and pedigree analysis involving a family known as the blue Fugates of Troublesome Creek. Enzyme tests indicated that the blue Fugates lacked the enzyme diaphorase, coded for by a gene on chromosome 22. The enzyme normally converts methemoglobin back to hemoglobin. The physician treated the disorder in a simple, but rather unconventional manner. He injected the Fugates with a dye called methylene blue! This unusual dye can donate electrons to other compounds, successfully converting the excess methemoglobin back into normal hemoglobin. The results were striking but immediate—the patient's skin quickly turned pink after treatment.

A pedigree analysis of the Fugate family indicated that the trait is common in the family because so many carried the recessive allele.

Cystic Fibrosis

Cystic fibrosis (CF) is the most common lethal genetic disease among Caucasians in the United States (Fig. 11.11). About 1 in 20 Caucasians is a carrier, and about 1 in 2,000 newborns has the disorder. CF patients exhibit a number of characteristic symptoms, the most obvious being extremely salty sweat. In children with CF, the mucus in the bronchial tubes and pancreatic ducts is particularly thick and viscous, interfering with the function of the lungs and pancreas. To ease breathing, the thick mucus in the lungs has to be loosened periodically, but still the lungs frequently become infected. The clogged pancreatic ducts prevent digestive enzymes from reaching the small intestine, and to improve digestion, patients take digestive enzymes mixed with applesauce before every meal.

Cystic fibrosis is caused by a defective chloride ion channel that is encoded by the *CFTR* allele on chromosome 7. Research has demonstrated that chloride ions (Cl^-) fail to pass through the defective version of the CFTR chloride ion channel, which is located on the plasma membrane. Ordinarily, after chloride ions have passed through the channel to the other side of the membrane, sodium ions (Na^+) and water follow. It is believed that lack of water is the cause of the abnormally thick mucus in the bronchial tubes and pancreatic ducts.

In the past few years, new treatments have raised the average life expectancy for CF patients to as much as 35 years of age. It is hoped that other novel treatments, such as gene therapy, may be able to correct the defect by placing a normal copy of the gene in patients to replace the faulty ones. To explain the persistence of the mutated *CFTR* allele in a population, it has been suggested that those heterozygous for CF are less likely to die from potentially fatal diseases, such as cholera.

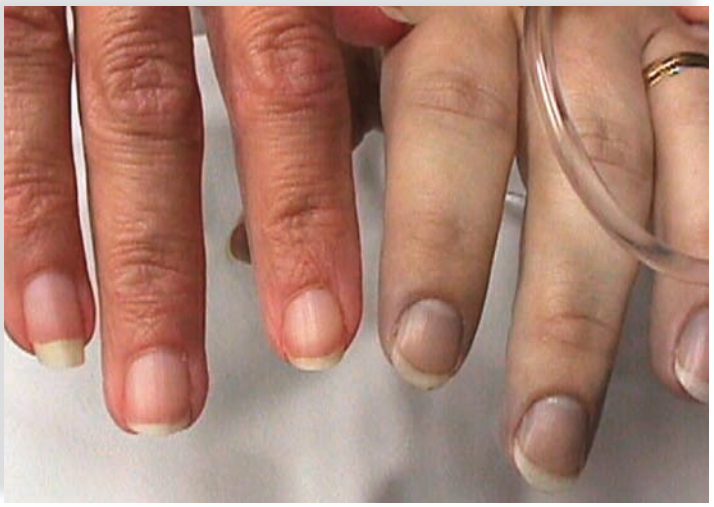


FIGURE 11.10 Methemoglobinemia.

The hands of the woman on the right appear blue due to chemically induced methemoglobinemia.

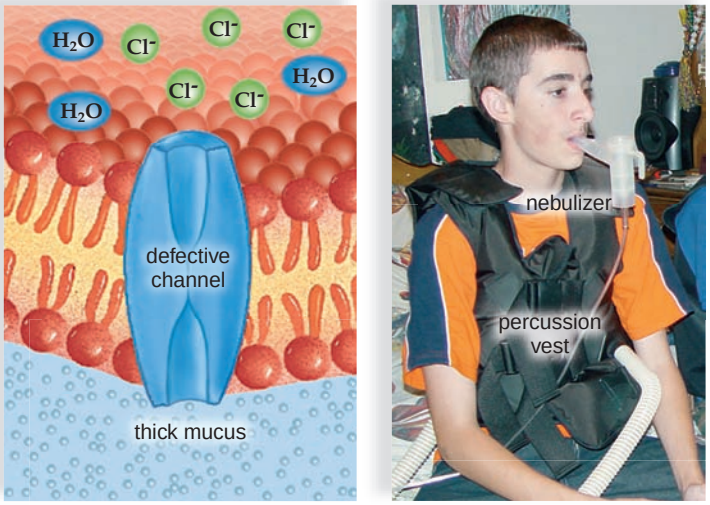


FIGURE 11.11 Cystic fibrosis.

Cystic fibrosis is due to a faulty protein that is supposed to regulate the flow of chloride ions into and out of cells through a channel protein.

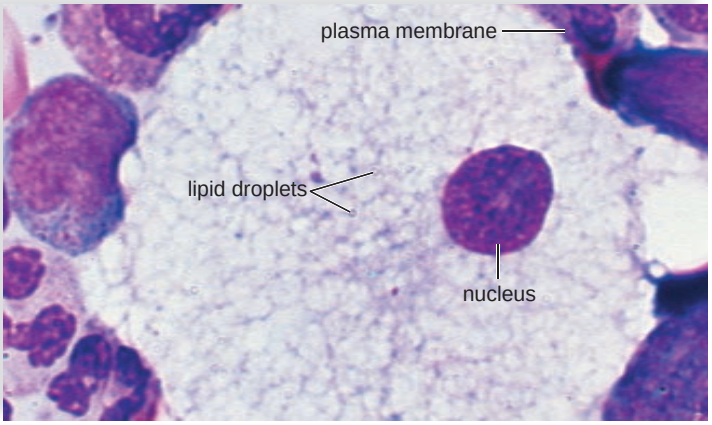


FIGURE 11.12 Niemann-Pick disease.

Persons with Niemann-Pick disease accumulate lipid droplets containing sphingomyelin within the cytoplasm of cells in the liver, spleen, and lymph nodes.

Niemann-Pick Disease

In infants, a persistent jaundice, feeding difficulties, an enlarged abdomen, and pronounced mental retardation may signal to a medical professional that the child has Niemann-Pick disease.

Type A and B forms of Niemann-Pick disease are caused by defective versions of the same gene located on chromosome 11. This gene codes for acid sphingomyelinase, an enzyme that normally breaks down a lipid called sphingomyelin. Affected individuals accumulate lipid droplets within cells of the liver, lymph nodes, and spleen (Fig. 11.12). The abnormal accumulation of lipids causes enlargement of the abdomen, one of the hallmarks of the disease. In more severe cases, the lipids build up within the brain as well, causing the severe neurological problems characteristic of type A. Although both A and B forms of Niemann-Pick disease are caused by defective versions of the same gene, type B is the milder form because the protein product of its allele has some activity, while the protein product of the type A allele is totally inactive.

Autosomal Dominant Disorders

A number of autosomal dominant disorders have been identified in humans. Two relatively well-known autosomal dominant disorders include osteogenesis imperfecta and hereditary spherocytosis.

Osteogenesis Imperfecta

Osteogenesis [L. *os*, bone, *genesis*, origin] imperfecta is an autosomal dominant genetic disorder that results in weakened, brittle bones. Although there are at least nine types of the disorder, most are linked to mutations in two genes necessary to the synthesis of a type I collagen—one of the most abundant proteins in the human body. Collagen has many roles, including providing strength and rigidity to bone and forming the framework for most of the body's tissues. Osteogenesis imperfecta leads to a defective collagen I that causes the bones to be brittle and weak. Because the mutant collagen can cause structural defects even when combined with normal collagen I, osteogenesis imperfecta is generally considered to be dominant.

Osteogenesis imperfecta, which has an incidence of approximately 1 in 5,000 live births, affects all racial groups similarly, and has been documented as long as 300 years ago. Because he was often carried into battle on a shield and was known as Ivar, the Boneless, some historians suspect that the Viking chieftain, Ivar Ragnarsson had the condition. In most cases, the diagnosis is made in young children who visit the emergency room frequently due to broken bones. Some children with the disorder have an unusual blue tint in the sclera, the white portion of the eye, reduced skin elasticity, weakened teeth, and occasionally heart valve abnormalities. Currently, the disorder is treatable with a number of drugs that help to increase bone mass, but these drugs must be taken long-term.

Hereditary Spherocytosis

Hereditary spherocytosis is an autosomal dominant genetic blood disorder that results from a defective copy of the ankyrin-1 gene found on chromosome 8. The protein encoded by this gene serves as a structural component of red blood cells, and is responsible for maintaining their disk-like shape. The abnormal spherocytosis protein is unable to perform its usual function, causing the affected person's red blood cells to adopt a spherical shape. As a result, the abnormal cells are fragile and burst easily, especially under osmotic stress. Enlargement of the spleen is also commonly seen in people with the disorder.

With an incidence of approximately 1 in 5,000, hereditary spherocytosis is one of the most common hereditary blood disorders. Roughly one-fourth of these cases result from new mutations and are not inherited from either parent. Hereditary spherocytosis exhibits incomplete penetrance, so not all individuals who inherit the mutant allele will exhibit the trait. The cause of incomplete penetrance in these cases and others remains poorly understood.

Check Your Progress 11.2E

1. What is the genotype of the child in Figure 11.11? What are the genotypes of his parents if neither parent has cystic fibrosis? (Use this key: C = normal; c = cystic fibrosis)
2. What is the chance that the parents in the above problem will have a child with cystic fibrosis?
3. What is the genotype of the woman in Figure 11B*a* if she is heterozygous? What is the genotype of a husband who is homozygous recessive? (Use this key: H = Huntington; h = unaffected)
4. What is the probability that the parents in the above problem will have a child with Huntington disease?

science focus

Testing for Genetic Disorders

Many human genetic disorders such as Huntington disease and cystic fibrosis are the result of inheriting faulty genes. Huntington disease (Fig. 11B*a*) is a devastating neurological disease caused by the inheritance of a single dominant allele, while cystic fibrosis, being a recessive disorder, requires the inheritance of two recessive alleles. Many adults want to be tested to see if they have a particular genetic disease or if they are a carrier for a disease. A carrier appears to be normal but is capable of passing on the recessive allele for the disorder. When you are tested for a genetic disorder, what does the technician test? Your DNA, of course! Tests have been developed that can detect a particular sequence of bases, and this sequence tells whether you have the genetic disorder.

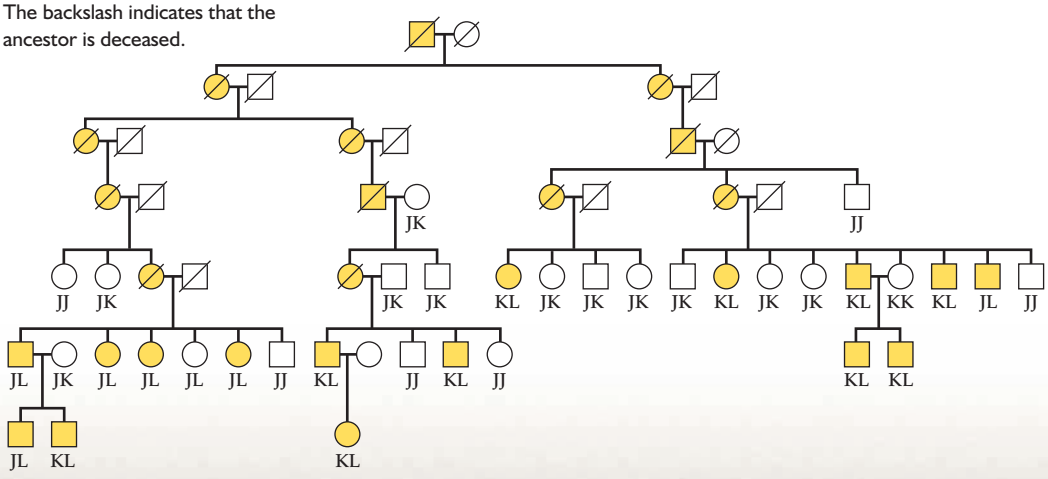
When researchers set out to develop a test for Huntington disease, they first obtained multiple **family pedigrees,** such as the one shown in Figure 11B*b*. This pedigree meets the requirements for a dominant allele: Every individual who is affected (shaded box or circle) has a parent who is also affected, heterozygotes are affected, and both males and females are affected in equal numbers. Each offspring of an affected individual has a 50% chance of getting the faulty gene and having Huntington disease, which doesn't appear until later in life.

The letters under the square or circle mean the individual has undergone a blood test that resulted in an analysis of their DNA. A computer was employed to search the DNA of all these individuals for similar base sequences. The computer found that a large number of individuals either had a sequence designated as J, K, or L. Only the sequence of bases designated as L appears in all the individuals with Huntington disease. Is this sequence a part of the gene for Huntington or is it in a gene that is linked to Huntington? Apparently, it is not in the gene for Huntington because at least one individual has the sequence but does not have Huntington disease. Several alleles can occur on the same chromosome, and these alleles are said to be linked. Linked alleles tend to go into the same gamete together, and this is the reason that alleles must be on separate chromosomes for the law of independent assortment to hold. Still, even genes that are closely linked can undergo crossing-over and become unlinked on occasion. Testable sequences that are closely linked to that of the faulty gene are called genetic markers, and genetic markers can be used as tests for genetic disorders, such as Huntington disease.

Association studies are another way for researchers to find possible sequences that indicate someone has a genetic disorder. During an association study, the DNA of a diverse sample of the general population is tested to find similar DNA sequences. If, for example, it turns out that many people who have type 2 diabetes have a particular sequence, this sequence might be used as a genetic marker for type 2 diabetes. With the advent of the human genome project, which resulted in the sequencing of all the bases in human DNA, it has been possible to successfully identify many genes that were formerly only tied to a particular chromosome by use of markers.

The mapping of disorders to genes within the human genome, while often painstaking and difficult to accomplish, has yielded much valuable information to the scientific community. The information can be used in prenatal genetic testing, for diagnosis of the disorder in individuals before symptoms occur, and for carrier testing in the case of disorders that are recessive. This information can be used to further understand the origin, progression, and pathology of the disorder, which may also lead to novel treatment methods. New techniques and technologies have greatly accelerated this process, but the tried and true methods of family pedigrees and association studies are still the primary techniques used by geneticists in pursuing the cure for many human genetic ailments.

a.



b.

FIGURE 11B Blood sample testing.
a. Huntington disease is a devastating neurological condition. **b.** In order to develop a test for Huntingon disease, researchers used white blood cells to discover that a particular sequence of DNA bases (L) is always present when a person has Huntington. The pedigree chart shows that (L) is not present unless an ancestor had Huntington disease. The backslash indicates that the ancestor is deceased.

11.3 Extending the Range of Mendelian Genetics

Mendelian genetics can also be applied to complex patterns of inheritance, such as multiple alleles, incomplete dominance, pleiotropy, and polygenic inheritance.

Multiple Allelic Traits

When a trait is controlled by **multiple alleles,** the gene exists in several allelic forms. For example, while a person's ABO blood type is controlled by a single gene pair, there are three possible alleles that determine the blood type. These alleles determine the presence or absence of antigens on red blood cells.

I^A = A antigen on red blood cells
I^B = B antigen on red blood cells
i = Neither A nor B antigen on red blood cells

The possible phenotypes and genotypes for blood type are as follows:

Phenotype	*Genotype*
A	I^AI^A, I^Ai
B	I^BI^B, I^Bi
AB	I^AI^B
O	ii

The inheritance of the ABO blood group in humans is also an example of **codominance** because both I^A and I^B are fully expressed in the presence of the other. Therefore, a person inheriting one of each of these alleles will have type AB blood. On the other hand, both I^A and I^B are dominant over i. Therefore, there are two possible genotypes for type A blood, and two possible genotypes for type B blood. Use a Punnett square to confirm that reproduction between a heterozygote with type A blood and a heterozygote with type B blood can result in any one of the four blood types. Such a cross makes it clear that an offspring can have a different blood type from either parent, and for this reason, DNA fingerprinting is now used to identify the parents of an individual instead of blood type.

Incomplete Dominance and Incomplete Penetrance

Incomplete dominance is exhibited when the heterozygote has an intermediate phenotype between that of either homozygote. In a cross between a true-breeding, red-flowered four-o'clock strain and a true-breeding, white-flowered strain, the offspring have pink flowers. But this is not an example of the blending inheritance. When the pink plants self-pollinate, the offspring have a phenotypic ratio of 1 red-flowered : 2 pink-flowered : 1 white-flowered plant. The reappearance of the three phenotypes in this generation makes it clear that we are still dealing with a single pair of alleles (Fig. 11.13).

Incomplete dominance in four-o'clocks can be explained in this manner: A double dose of pigment results in red flowers; a single dose of pigment results in pink flowers; and because white flowers produce no pigment, the flowers are white.

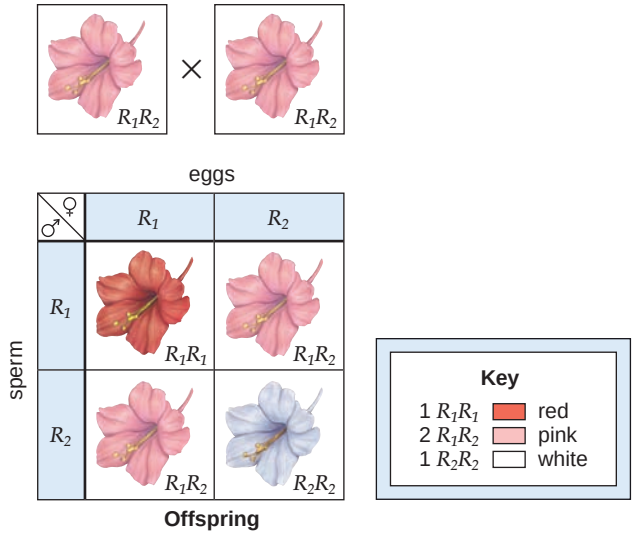


FIGURE 11.13 Incomplete dominance.

When pink four-o'clocks self-pollinate, the results show three phenotypes. This is only possible if the pink parents had an allele for red pigment (R_1) and an allele for no pigment (R_2). Note that alleles involved in incomplete dominance are both given a capital letter.

Human Examples of Incomplete Dominance

In humans, familial hypercholesterolemia (FH) is an example of incomplete dominance. An individual with two alleles for this disorder develops fatty deposits in the skin and tendons and may have a heart attack as a child. An individual with one normal allele and one *FH* allele may suffer a heart attack as a young adult, and an individual with two normal alleles does not have the disorder.

Perhaps the inheritance pattern of other human disorders should be considered one of incomplete dominance. To detect the carriers of cystic fibrosis, for example, it is customary to determine the amount of cellular activity of the gene. When the activity is one-half that of the dominant homozygote, the individual is a carrier, even though the individual does not exhibit the genetic disease. In other words, at the level of gene expression, the homozygotes and heterozygotes do differ in the same manner as four-o'clock plants.

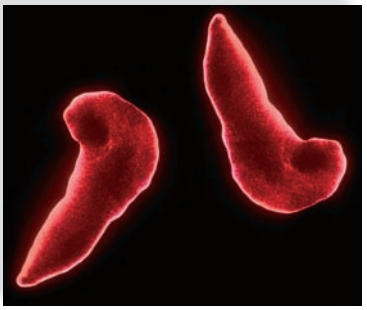
In some cases, a dominant allele may not always lead to the dominant phenotype in a heterozygote, even when the alleles show a true dominant/recessive relationship. The dominant allele in this case does not always determine the phenotype of the individual, so we describe these traits as showing **incomplete penetrance.** Many dominant alleles exhibit varying degrees of penetrance.

The best-known example is polydactyly, the presence of one or more extra digits on hands, feet, or both. Polydactyly is inherited in an autosomal dominant manner; however, not all individuals who inherit the dominant allele will exhibit the trait. The reasons for this are not clear, but expression of polydactyly may require additional environmental factors or be influenced by other genes, as discussed again later.

Pleiotropic Effects

Pleiotropy occurs when a single mutant gene affects two or more distinct and seemingly unrelated traits. For example, persons with Marfan syndrome have disproportionately long arms, legs, hands, and feet; a weakened aorta; poor eyesight; and other characteristics (Fig. 11.14). All of these characteristics are due to the production of abnormal connective tissue. Marfan syndrome has been linked to a mutated gene (FBN_1) on chromosome 15 that ordinarily specifies a functional protein called fibrillin. Fibrillin is essential for the formation of elastic fibers in connective tissue. Without the structural support of normal connective tissue, the aorta can burst, particularly if the person is engaged in a strenuous sport, such as volleyball or basketball. Flo Hyman may have been the best American woman volleyball player ever, but she fell to the floor and died at the age of only 31 because her aorta gave way during a game. Now that coaches are aware of Marfan syndrome, they are on the lookout for it among very tall basketball players. Chris Weisheit, whose career was cut short after he was diagnosed with Marfan syndrome, said, "I don't want to die playing basketball."

Many other disorders, including porphyria and sickle-cell disease, are examples of pleiotropic traits. Porphyria is caused by a chemical insufficiency in the production of hemoglobin, the pigment that makes red blood cells red. The symptoms of porphyria are photosensitivity, strong abdominal pain, port-wine-colored urine, and paralysis in the arms and legs. Many members of the British royal family in the late 1700s and early 1800s suffered from this disorder, which can lead to epileptic convulsions, bizarre behavior, and coma.

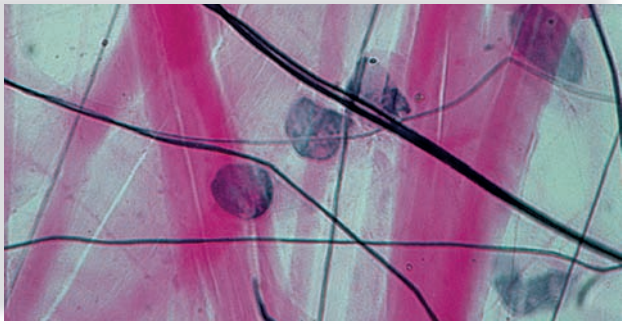


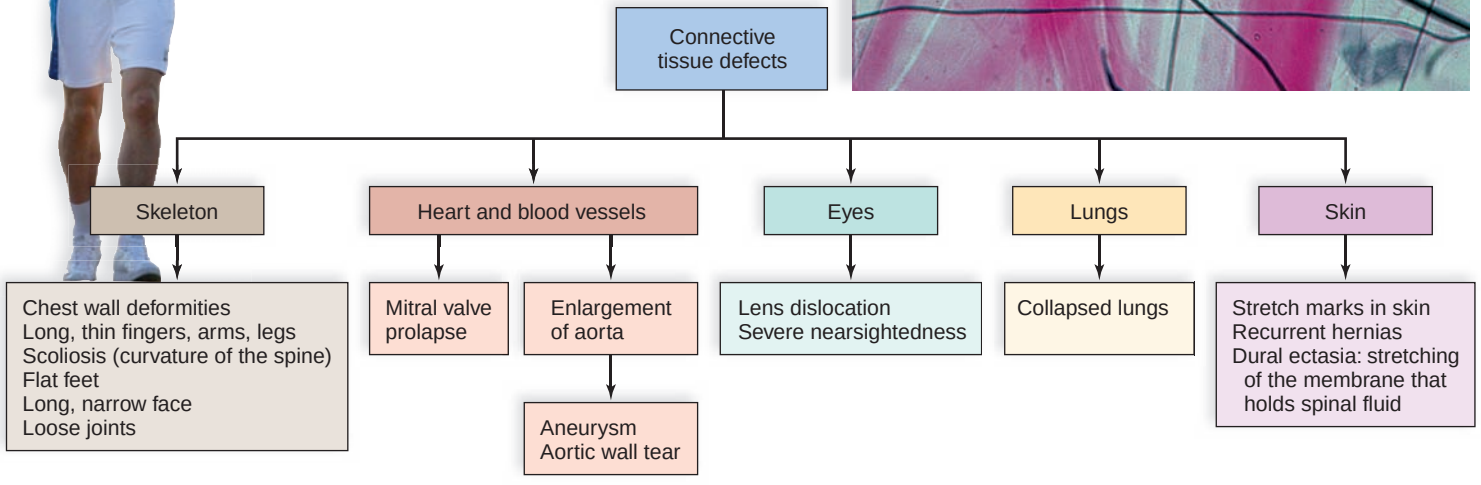
1,600×, colorized SEM
Sickled red blood cell

In a person suffering from sickle-cell disease (Hb^SHb^S), the cells are sickle-shaped. The underlying mutation is in a gene that codes for a type of polypeptide chain in hemoglobin. Of 146 amino acids, the mutation changes only one amino acid, but the result is a less soluble polypeptide chain that stacks up and causes red blood cells to be sickle-shaped. The abnormally shaped sickle cells slow down blood flow and clog small blood vessels. In addition, sickled red blood cells have a shorter life span than normal red blood cells. Affected individuals may exhibit a number of symptoms, including severe anemia, physical weakness, poor circulation, impaired mental function, pain and high fever, rheumatism, paralysis, spleen damage, low resistance to disease, and kidney and heart failure. All of thse effects are due to the tendency of sickled red blood cells to break down and to the resulting decreased oxygen-carrying capacity of the blood and the damage the body suffers as a result of the condition. Although sickle-cell disease is a devastating disorder, it provides heterozygous individuals with a survival advantage. People who have sickle-cell trait are resistant to the protozoan parasite that causes malaria. The parasite spends part of its life cycle in red blood cells feeding on hemoglobin, but it cannot complete its life cycle when sickle-shaped cells form and break down earlier than usual.

FIGURE 11.14 Marfan syndrome.

Marfan syndrome illustrates the multiple effects a single gene can have. Marfan syndrome is due to any number of defective connective tissue defects.





Polygenic Inheritance

Polygenic inheritance [Gk. *poly*, many; L. *genitus*, producing] occurs when a trait is governed by two or more sets of alleles. The individual has a copy of all allelic pairs, possibly located on many different pairs of chromosomes. Each dominant allele has a quantitative effect on the phenotype, and these effects are additive. Therefore, a population is expected to exhibit continuous phenotypic variations. In Figure 11.15, a cross between genotypes *AABBCC* and *aabbcc* yields F_1 hybrids with the genotype *AaBbCc*. A range of genotypes and phenotypes results in the F_2 generation that can be depicted as a bell-shaped curve (Fig. 11.15). **Multifactorial traits** are controlled by polygenes subject to environmental influences. We observed previously (see Fig. 6.9) that the coat color of a Siamese cat is darker in color at the ears, nose, paws, and tails because an enzyme involved in the production of melanin is active only at a low temperature. Similarly, multifactoral traits are controlled by polygenes subject to environmental affects.

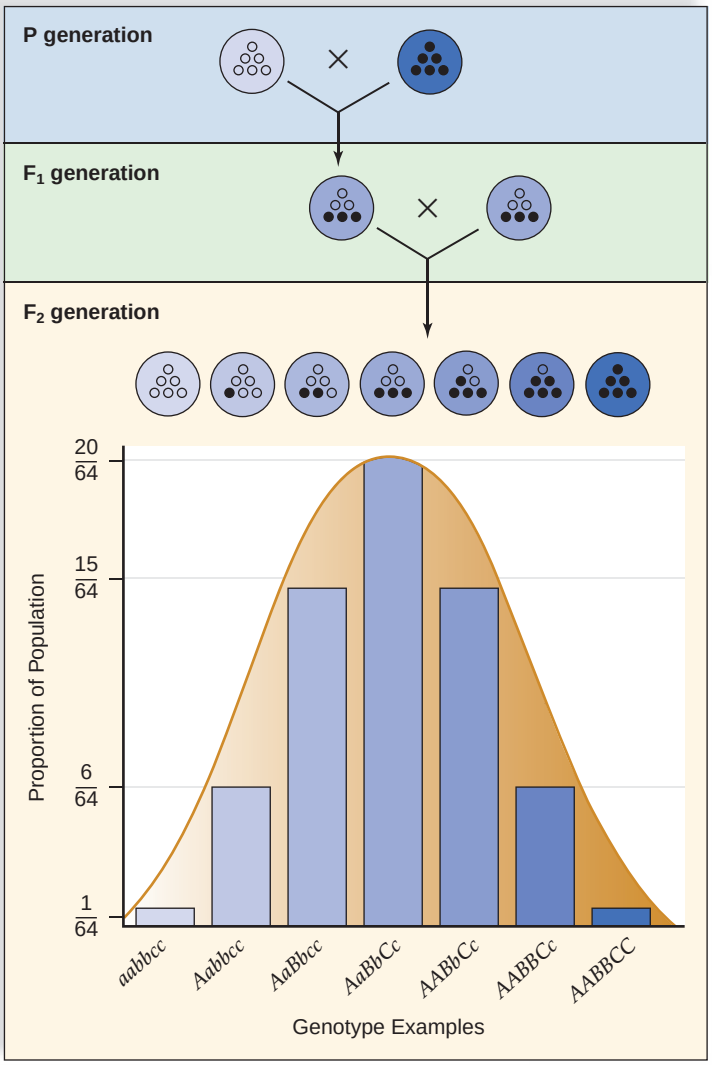


FIGURE 11.15 Polygenic inheritance.

In polygenic inheritance, a number of pairs of genes control the trait. *Above:* Black dots and intensity of blue shading stand for the number of dominant alleles. *Below:* Orange shading shows the degree of environmental influences.

Human Examples of Multifactorial Inheritance

Human skin color and height are examples of polygenic traits affected by the environment. For example, exposure to the sun can affect skin color and nutrition can affect human height. Just how many pairs of alleles control skin color is not known, but a range in colors can be explained on the basis of just two pairs when *each capital letter contributes equally to the pigment in the skin.*

Genotypes	*Phenotypes*
AABB	Very dark
AABb or *AaBB*	Dark
AaBb or *AAbb* or *aaBB*	Medium brown
Aabb or *aaBb*	Light
aabb	Very light

Eye color is also a polygenic trait. The amount of melanin deposited in the iris increases the darker color of the eye. Different eye colors from the brightest of blue to nearly black eyes are thought to be the result of two genes with alleles each interacting in an additive manner.

Many human disorders, such as cleft lip and/or palate, clubfoot, congenital dislocations of the hip, hypertension, diabetes, schizophrenia, and even allergies and cancers, are most likely due to the combined action of many genes plus environmental influences. In recent years, reports have surfaced that all sorts of behavioral traits, such as alcoholism, phobias, and even suicide, can be associated with particular genes. The relative importance of genetic and environmental influences on the phenotype can vary, but in some instances the role of the environment is clear. For example, cardiovascular disease is more prevalent among those whose biological or adoptive parents have cardiovascular disease. Can you suggest environmental reasons for this correlation, based on your study of Chapter 3?

Many investigators are trying to determine what percentage of various traits is due to nature (inheritance) and what percentage is due to nurture (the environment). Some studies use twins separated since birth, because if identical twins in different environments share the same trait, the trait is most likely inherited. Identical twins are more similar in their intellectual talents, personality traits, and levels of lifelong happiness than are fraternal twins separated at birth. Biologists conclude that all behavioral traits are partly heritable, and that genes exert their effects by acting together in complex combinations susceptible to environmental influences.

Check Your Progress 11.3A

1. If the inheritance pattern for a genetic disorder was exemplified by incomplete dominance, what would be the genotype of the heterozygote (see Fig. 11.13)?
2. A child with type O blood is born to a mother with type A blood. What is the genotype of the child? The mother? What are the possible genotypes of the father?
3. A polygenic trait is controlled by three different gene loci. Give seven genotypes among the offspring that will result in seven different phenotypes when *AaBbCc* is crossed with *AaBbCc*.

X-Linked Inheritance

The X and Y chromosomes in mammals determine the gender of the individual. Females are XX and males are XY. These chromosomes carry genes that control development and, in particular, if the Y chromosome contains an *SRY* gene, the embryo becomes a male. The term **X-linked** is used for genes that have nothing to do with gender, and yet they are carried on the X chromosome. The Y chromosome does not carry these genes and indeed carries very few genes. This type of inheritance was discovered in the early 1900s by a group at Columbia University, headed by Thomas Hunt Morgan. Morgan performed experiments with fruit flies, whose scientific name is *Drosophila melanogaster.* Fruit flies are even better subjects for genetic studies than garden peas. They can be easily and inexpensively raised in simple laboratory glassware: Females mate and then lay hundreds of eggs during their lifetimes; the generation time is short, taking only about ten days from egg to adult. Fruit flies have the same sex chromosome pattern as humans, and therefore Morgan's experiments with X-linked genes apply directly to humans.

Morgan's Experiment

Morgan took a newly discovered mutant male with white eyes and crossed it with a red-eyed female:

	♀		♂
P	red-eyed	×	white-eyed
F_1	red-eyed		red-eyed

From these results, he knew that red eyes are the dominant characteristic and white eyes are the recessive characteristic. He then crossed the F_1 flies. In the F_2 generation, there was the expected 3 red-eyed : 1 white-eyed ratio, but it struck him as odd that all of the white-eyed flies were males:

	♀		♂
$F_1 \times F_1$	red-eyed	×	red-eyed
F_2	red-eyed		1 red-eyed : 1 white-eyed

Obviously, a major difference between the male flies and the female flies was their sex chromosomes. Could it be possible that an allele for eye color was on the Y chromosome but not on the X? This idea could be quickly discarded because usually females have red eyes, and they have no Y chromosome. Perhaps an allele for eye color was on the X, but not on the Y, chromosome. Figure 11.16 indicates that this explanation would match the results obtained in the experiment. These results support the chromosome theory of inheritance by showing that the behavior of a specific allele corresponds exactly with that of a specific chromosome—the X chromosome in *Drosophila*.

Notice that X-linked alleles have a different pattern of inheritance than alleles that are on the autosomes because the Y chromosome is lacking for these alleles, and the inheritance of a Y chromosome cannot offset the inheritance of an X-linked recessive allele. For the same reason, males always receive an X-linked recessive mutant allele from the female parent—they receive the Y chromosome from the male parent, and therefore sex-linked recessive traits appear much more frequently in males than in females.

Solving X-Linked Genetics Problems

Recall that when solving autosomal genetics problems, the allele key and genotypes can be represented as follows:

Allele key	Genotypes
L = long wings	*LL, Ll, ll*
l = short wings	

When predicting inheritance of sex-linked traits, however, it is necessary to indicate the sex chromosomes of

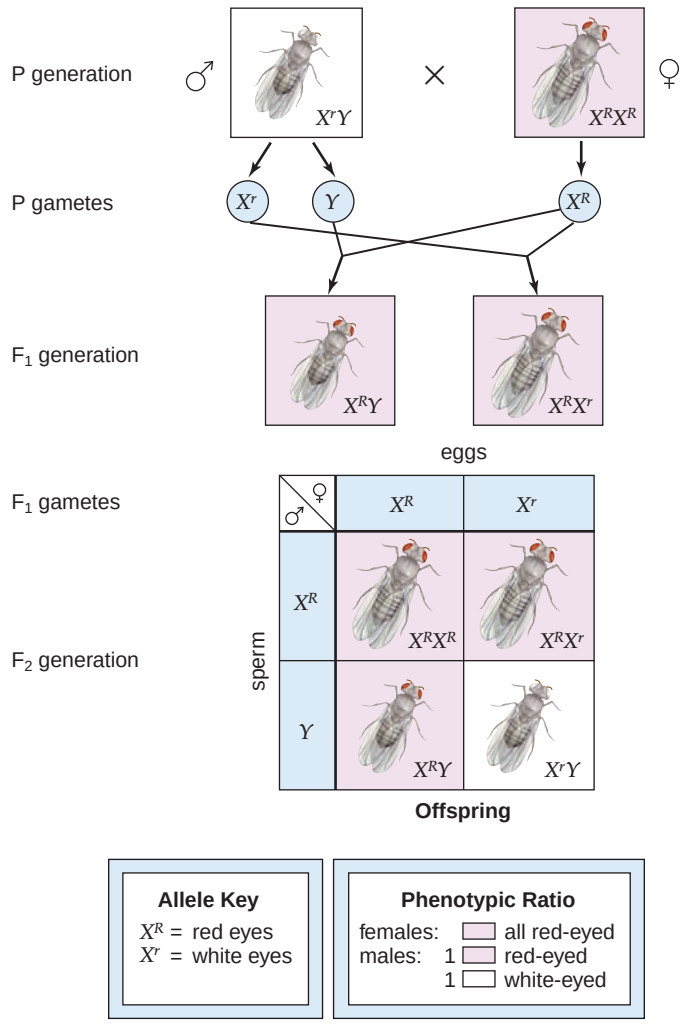


FIGURE 11.16 X-linked inheritance.

Once researchers deduced that the alleles for red/white eye color are on the X chromosome in *Drosophila,* they were able to explain their experimental results. Males with white eyes in the F_2 generation inherit the recessive allele only from the female parent; they receive a Y chromosome lacking the allele for eye color from the male parent.

each individual. As noted in Figure 11.16, however, the allele key for an X-linked gene shows an allele attached to the X:

Allele key
X^R = red eyes
X^r = white eyes

The possible genotypes in both males and females are as follows:

X^RX^R = red-eyed female
X^RX^r = red-eyed female
X^rX^r = white-eyed female
X^RY = red-eyed male
X^rY = white-eyed male

Notice that there are three possible genotypes for females but only two for males. Females can be heterozygous X^RX^r, in which case they are carriers. Carriers usually do not show a recessive abnormality, but they are capable of passing on a recessive allele for an abnormality. But unlike autosomal traits, males cannot be carriers for X-linked traits; if the dominant allele is on the single X chromosome, they show the dominant phenotype, and if the recessive allele is on the single X chromosome, they show the recessive phenotype. For this reason, males are considered **hemizygous** for X-linked traits, because a male only possesses one allele for the trait and, therefore, expresses whatever allele is present on the X chromosome.

We know that male fruit flies have white eyes when they receive the mutant recessive allele from the female parent. What is the inheritance pattern when females have white eyes? Females can only have white eyes when they receive a recessive allele from both parents.

Human X-Linked Disorders

Several X-linked recessive disorders occur in humans including color blindness, Menkes syndrome, muscular dystrophy, adrenoleukodystrophy, and hemophilia.

Color Blindness. In humans, the receptors for color vision in the retina of the eyes are three different classes of cone cells. Only one type of pigment protein is present in each class of cone cell; there are blue-sensitive, red-sensitive, and green-sensitive cone cells. The allele for the blue-sensitive protein is autosomal, but the alleles for the red- and green-sensitive pigments are on the X chromosome. About 8% of Caucasian men have red-green color blindness. Most of these see brighter greens as tans, olive greens as browns, and reds as reddish browns. A few cannot tell reds from greens at all. They see only yellows, blues, blacks, whites, and grays.

Pedigrees can also reveal the unusual inheritance pattern seen in sex-linked traits. For example, the pedigree in Figure 11.17 shows the usual pattern of inheritance for color blindness. More males than females have the trait because recessive alleles on the X chromosome are expressed in males. The disorder often passes from grandfather to grandson through a carrier daughter.

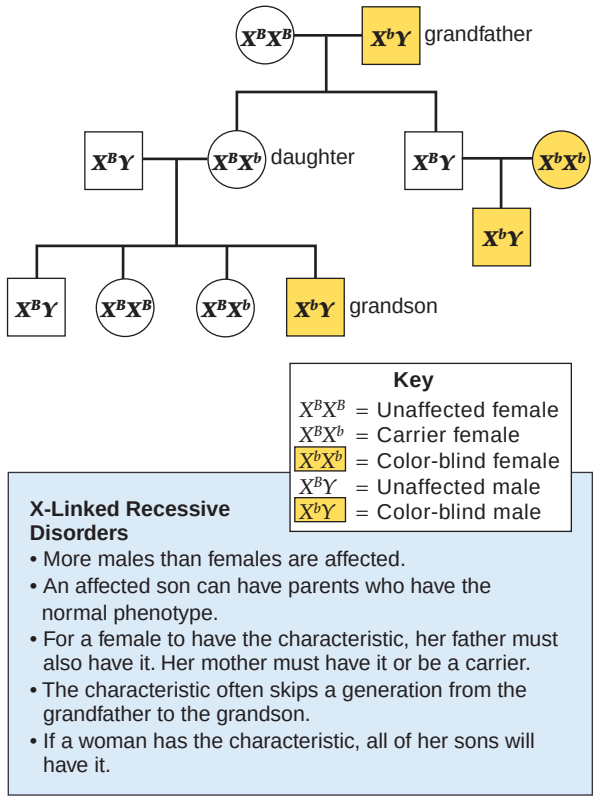


FIGURE 11.17 X-linked recessive pedigree.

This pedigree for color blindness exemplifies the inheritance pattern of an X-linked recessive disorder. The list gives various ways of recognizing the X-linked recessive pattern of inheritance.

Menkes Syndrome. Menkes syndrome, or kinky hair syndrome, is caused by a defective allele on the X chromosome. Normally, the gene product controls the movement of the metal copper in and out of cells. The symptoms of Menkes syndrome are due to accumulation of copper in some parts of the body, and the lack of the metal in other parts.

Symptoms of Menkes syndrome include poor muscle tone, seizures, abnormally low body temperature, skeletal anomalies, and the characteristic brittle, steely hair associated with the disorder. Although the condition is relatively rare, affecting approximately 1 in 100,000, mostly males, the prognosis for people with Menkes syndrome is poor, and most individuals die within the first few years of life. In recent years, some people with Menkes syndrome have been treated with injections of copper directly underneath the skin, but with mixed results, and treatment must begin very early in life to be effective.

Muscular Dystrophy. Muscular dystrophy, as the name implies, is characterized by a wasting away of the mus-

cles. The most common form, Duchenne muscular dystrophy, is X-linked and occurs in about 1 out of every 3,600 male births (Fig. 11.18). Symptoms, such as waddling gait, toe walking, frequent falls, and difficulty in rising, may appear as soon as the child starts to walk. Muscle weakness intensifies until the individual is confined to a wheelchair. Death usually occurs by age 20; therefore, affected males are rarely fathers. The recessive allele remains in the population through passage from carrier mother to carrier daughter.

The allele for Duchenne muscular dystrophy has been isolated, and it was discovered that the absence of a protein called dystrophin causes the disorder. Much investigative work determined that dystrophin is involved in the release of calcium from the sarcoplasmic reticulum in muscle fibers. The lack of dystrophin causes calcium to leak into the cell, which promotes the action of an enzyme that dissolves muscle fibers. When the body attempts to repair the tissue, fibrous tissue forms, and this cuts off the blood supply so that more and more cells die.

A test is now available to detect carriers of Duchenne muscular dystrophy. Also, various treatments have been tried. Immature muscle cells can be injected into muscles, and for every 100,000 cells injected, dystrophin production occurs in 30–40% of muscle fibers. The allele for dystrophin has been inserted into thigh muscle cells, and about 1% of these cells then produced dystrophin.

Adrenoleukodystrophy. Adrenoleukodystrophy, or ALD, is an X-linked recessive disorder due to the failure of a carrier protein to move either an enzyme or very long chain fatty acid (24–30 carbon atoms) into peroxisomes. As a result, these fatty acids are not broken down, and they accumulate inside the cell and the result is severe nervous system damage.

Children with ALD fail to develop properly after age 5, lose adrenal gland function, exhibit very poor coordination, and show a progressive loss of hearing, speech, and vision. The condition is usually fatal, with no known cure, but the onset and severity of symptoms in patients not yet showing symptoms may be mitigated by treatment with a mixture of lipids derived from olive oil. The disease was made famous by the 1992 movie *Lorenzo's Oil*, detailing a mother's and father's determination to devise a treatment for their son who was suffering from ALD.

Hemophilia. About 1 in 10,000 males is a hemophiliac. There are two common types of hemophilia: Hemophilia A is due to the absence or minimal presence of a clotting factor known as factor VIII, and hemophilia B is due to the absence of clotting factor IX. Hemophilia is called the bleeder's disease because the affected person's blood either does not clot or clots very slowly. Although hemophiliacs bleed externally after an injury, they also bleed internally, particularly around joints. Hemorrhages can be stopped with transfusions of fresh blood (or plasma) or concentrates of the clotting protein. Also, clotting factors are now available as biotechnology products.

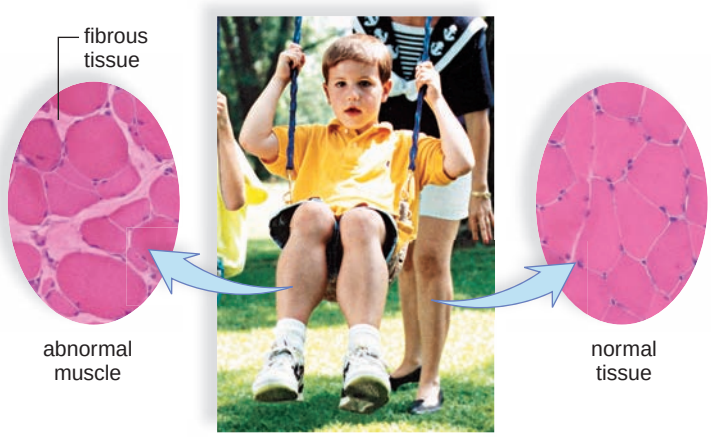


FIGURE 11.18 Muscular dystrophy.

In muscular dystrophy, an X-linked recessive disorder, calves enlarge because fibrous tissue develops as muscles waste away, due to lack of the protein dystrophin.

At the turn of the century, hemophilia was prevalent among the royal families of Europe, and all of the affected males could trace their ancestry to Queen Victoria of England. Of Queen Victoria's 26 grandchildren, four grandsons had hemophilia and four granddaughters were carriers. Because none of Queen Victoria's relatives were affected, it seems that the faulty allele she carried arose by mutation either in Victoria or in one of her parents. Her carrier daughters Alice and Beatrice introduced the allele into the ruling houses of Russia and Spain, respectively. Alexis, the last heir to the Russian throne before the Russian Revolution, was a hemophiliac. There are no hemophiliacs in the present British royal family because Victoria's eldest son, King Edward VII, did not receive the allele.

Check Your Progress 11.3B

1. In *Drosophila*, if a homozygous red-eyed female is crossed with a red-eyed male, what would be the possible genotypes of their offspring?
2. A woman is color-blind. **a.** What are the chances that her sons will be color-blind? **b.** If she is married to a man with normal vision, what are the chances that her daughters will be color-blind? **c.** Will be carriers?
3. In a cross between a brown-haired female and a black-haired male, all male offspring have brown hair and all female offspring have black hair. What is the genotype of all individuals involved, assuming X-linkage?

Connecting the Concepts

A good experimental design and a bit of luck allowed Mendel to discover his laws of inheritance.

Although humans do not usually produce a large number of offspring, it has been possible to conclude that Mendel's laws do apply to humans in many instances. Good historical records of inheritance in large families, such as Mormon families, have allowed researchers to show that a number of human genetic disorders are indeed controlled by a single allelic pair. Such disorders include methemoglobinemia, cystic fibrosis, osteogenesis imperfecta, hereditary spherocytosis, and Marfan syndrome.

Mendel was lucky in that he chose to study an organism, namely the garden pea, whose observable traits are often determined by a single allelic pair. In most cases, however, traits are often determined by several genes or are affected by additional factors, such as the environment. These other types of inheritance patterns that differ from simple Mendelian inheritance are also discussed in this chapter.

The work of Morgan and others showed that the sex chromosomes contain genes unrelated to gender. Geneticists later discovered that some human genetic diseases, such as hemophilia, are caused by faulty genes on the X chromosome. With the help of Mendelian genetics, pedigree analysis, and statistics, scientists have been able to link many human diseases to specific genes on certain chromosomes. This knowledge later fueled an intense interest in deciphering exactly how these faulty genes could lead to such devastating diseases. As you will learn in the next chapter, genes on chromosomes direct the production of proteins in the cytoplasm of a cell through an RNA intermediate. It is the activity, or inactivity, of these proteins that leads to the observed phenotypes.

summary

11.1 Gregor Mendel

Gregor Mendel used the garden pea as the subject in his genetic studies. In contrast to preceding plant breeders, his study involved nonblending traits of the garden pea. Mendel applied mathematics, followed the scientific method very closely, and kept careful records. Therefore, he arrived at a particulate theory of inheritance, effectively disproving the blending theory of inheritance.

11.2 Mendel's Laws

When Mendel crossed heterozygous plants with other heterozygous plants, he found that the recessive phenotype reappeared in about ¼ of the F_2 plants; there was a 3:1 phenotypic ratio. This allowed Mendel to propose his law of segregation, which states that the individual has two factors for each trait, and the factors segregate into the gametes.

Mendel conducted two-trait crosses, in which the F_1 individuals showed both dominant characteristics, but there were four phenotypes among the F_2 offspring. (The actual phenotypic ratio was 9:3:3:1.) This allowed Mendel to deduce the law of independent assortment, which states that the members of one pair of factors separate independently of those of another pair. Therefore, all possible combinations of parental factors can occur in the gametes.

The laws of probability can be used to calculate the expected phenotypic ratio of a cross. A large number of offspring must be counted in order to observe the expected results, and to ensure that all possible types of sperm have fertilized all possible types of eggs, as is done in a Punnett square. The Punnett square uses the product law of probability to arrive at possible genotypes among the offspring, and then the sum law can be used to arrive at the phenotypic ratio.

Mendel also crossed the F_1 plants having the dominant phenotype with homozygous recessive plants. The 1:1 results indicated that the recessive factor was present in these F_1 plants (i.e., that they were heterozygous). Today, we call this a testcross, because it is used to test whether an individual showing the dominant characteristic is homozygous dominant or heterozygous. The two-trait testcross allows an investigator to test whether an individual showing two dominant characteristics is homozygous dominant for both traits or for one trait only, or is heterozygous for both traits.

Studies have shown that many human traits and genetic disorders can be explained on the basis of simple Mendelian inheritance. When studying human genetic disorders, biologists often construct pedigrees to show the pattern of inheritance of a characteristic within a family. The particular pattern indicates the manner in which a characteristic is inherited. Sample pedigrees for autosomal recessive and autosomal dominant patterns appear in Figures 11.8 and 11.9.

11.3 Extending the Range of Mendelian Genetics

Other patterns of inheritance have been discovered since Mendel's original contribution. For example, some genes have multiple alleles, although each individual organism has only two alleles, as in the inheritance of blood type in human beings. Inheritance of blood type also illustrates codominance. With incomplete dominance, the F_1 individuals are intermediate between the parent phenotypes; this does not support the blending theory because the parent phenotypes reappear in F_2. With incomplete penetrance, some traits that are dominant may not be expressed due to unknown reasons.

In pleiotropy, one gene has multiple effects as with Marfan syndrome and sickle-cell disease. Polygenic traits are controlled by several genes that have an additive effect on the phenotype, resulting in quantitative variations. A bell-shaped curve is seen because environmental influences bring about many intervening phenotypes, as in the inheritance of height in human beings. Skin color and eye color are also examples of multifactorial inheritance (polygenes plus the environment).

In *Drosophila*, as in humans, the sex chromosomes determined the sex of the individual, with XX being female and XY being male. Experimental support for the chromosome theory of inheritance came when Morgan and his group were able to determine that the gene for a trait unrelated to sex determination, the white-eyed allele in *Drosophila*, is on the X chromosome.

Alleles on the X chromosome are called X-linked alleles. Therefore, when doing X-linked genetics problems, it is the custom to indicate the sexes by using sex chromosomes and to indicate the alleles by superscripts attached to the X. The Y is blank because it does not carry these genes. Color blindness, Menkes syndrome, adrenoleukodystrophy, and hemophilia are X-linked recessive disorders in humans.

understanding the terms

allele 193
autosome 198
carrier 198
codominance 202
dihybrid cross 194
dominant allele 193
family pedigree 201
gene locus 193
genotype 193
hemizygous 206
heterozygous 193
homozygous 193
incomplete dominance 202
incomplete penetrance 202
monohybrid cross 192
multifactorial trait 204
multiple alleles 202
phenotype 193
pleiotropy 203
polygenic inheritance 204
Punnett square 196
recessive allele 193
testcross 197
X-linked 205

Match the terms to these definitions:

a. _____ Allele that exerts its phenotypic effect only in the homozygote; its expression is masked by a dominant allele.
b. _____ Alternative form of a gene that occurs at the same locus on homologous chromosomes.
c. _____ Polygenic trait that is subject to environmental affects.
d. _____ Cross between an individual with the dominant phenotype and an individual with the recessive phenotype to see if the individual with the dominant phenotype is homozygous or heterozygous.
e. _____ Genes of an organism for a particular trait or traits; for example, *BB* or *Aa*.

reviewing this chapter

1. How did Mendel's procedure differ from that of his predecessors? What is his theory of inheritance called? 190
2. How does the F_2 of Mendel's one-trait cross refute the blending concept of inheritance? Using Mendel's one-trait cross as an example, trace his reasoning to arrive at the law of segregation. 190–92
3. Using Mendel's two-trait cross as an example, trace his reasoning to arrive at the law of independent assortment. 194
4. What are the two laws of probability, and how do they apply to a Punnett square? 196
5. What is a testcross, and when is it used? 197
6. How might you distinguish an autosomal dominant trait from an autosomal recessive trait when viewing a pedigree? 198
7. For autosomal recessive disorders, what are the chances of two carriers having an affected child? 199–200
8. For most autosomal dominant disorders, what are the chances of a heterozygote and a normal individual having an affected child? 200
9. Explain inheritance by multiple alleles. List the human blood types, and give the possible genotypes for each. 202
10. Explain the inheritance of incompletely dominant alleles and why this is not an example of blending inheritance. 202
11. Explain why traits controlled by polygenes show continuous variation and produce a distribution in the F_2 generation that follows a bell-shaped curve. 204
12. How do you recognize a pedigree for an X-linked recessive allele in human beings? 205–6

testing yourself

Choose the best answer for each question. For questions 1–4, match each item to those in the key.

KEY:

a. 3:1
b. 9:3:3:1
c. 1:1
d. 1:1:1:1
e. 3:1:3:1

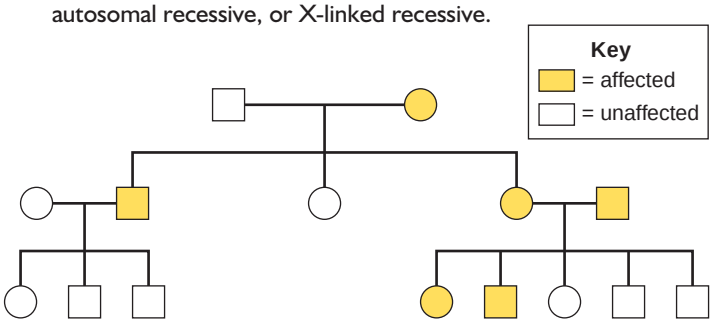
1. *TtYy* × *TtYy*
2. *Tt* × *Tt*
3. *Tt* × *tt*
4. *TtYy* × *ttyy*
5. Which of these could be a normal gamete?
   a. *GgRr*
   b. *GRr*
   c. *Gr*
   d. *GgR*
   e. None of these are correct.
6. Which of these properly describes a cross between an individual who is homozygous dominant for hairline but heterozygous for finger length and an individual who is recessive for both characteristics? (*W* = widow's peak, *w* = straight hairline, *S* = short fingers, *s* = long fingers)
   a. *WwSs* × *WwSs*
   b. *WWSs* × *wwSs*
   c. *Ws* × *ws*
   d. *WWSs* × *wwss*
7. In peas, yellow seed (*Y*) is dominant over green seed (*y*). In the F_2 generation of a monohybrid cross that begins when a dominant homozygote is crossed with a recessive homozygote, you would expect
   a. three plants with yellow seeds to every plant with green seeds.
   b. plants with one yellow seed for every green seed.
   c. only plants with the genotype *Yy*.
   d. only plants that produce yellow seeds.
   e. Both c and d are correct.
8. In humans, pointed eyebrows (*B*) are dominant over smooth eyebrows (*b*). Mary's father has pointed eyebrows, but she and her mother have smooth. What is the genotype of the father?
   a. *BB*
   b. *Bb*
   c. *bb*
   d. *BbBb*
   e. Any one of these is correct.
9. In guinea pigs, smooth coat (*S*) is dominant over rough coat (*s*), and black coat (*B*) is dominant over white coat (*b*). In the cross *SsBb* × *SsBb*, how many of the offspring will have a smooth black coat on average?
   a. 9 only
   b. about $^9/_{16}$
   c. $^1/_{16}$
   d. $^6/_{16}$
   e. $^2/_6$
10. In horses, *B* = black coat, *b* = brown coat, *T* = trotter, and *t* = pacer. A black trotter that has a brown pacer offspring would have which of the following genotypes?
    a. *BT*
    b. *BbTt*
    c. *bbtt*
    d. *BBtt*
    e. *BBTT*

11. In tomatoes, red fruit (*R*) is dominant over yellow fruit (*r*), and tallness (*T*) is dominant over shortness (*t*). A plant that is *RrTT* is crossed with a plant that is *rrTt*. What are the chances of an offspring possessing both recessive traits?
    a. none
    b. ½
    c. ¼
    d. ¾
12. In the cross *RrTt* × *rrtt*,
    a. all the offspring will be tall with red fruit.
    b. 75% (¾) will be tall with red fruit.
    c. 50% (½) will be tall with red fruit.
    d. 25% (¼) will be tall with red fruit.
13. A boy is color-blind (X-linked recessive) and has a straight hairline (autosomal recessive). Which could be the genotype of his mother?
    a. *bbww*
    b. X^bYWw
    c. bbX^wX^w
    d. X^BX^bWw
    e. X^wX^wBb
14. Which of the following would you *not* find in a pedigree when a male has an X-linked recessive disorder?
    a. Neither parent has the disorder.
    b. Only males in the pedigree have the disorder.
    c. Only females in the pedigree have the disorder.
    d. The sons of a female with the disorder will all have the disorder.
    e. Both a and c would not be seen.

For questions 15–17, match the statements to the items in the key.

KEY:
a. multiple alleles
b. polygenes
c. pleiotropic gene

15. People with sickle cell disease have many cardiovascular complications.
16. Although most people have an IQ of about 100, IQ generally ranges from about 50 to 150.
17. In humans, there are three possible alleles at the chromosomal locus that determine blood type.
18. Alice and Henry are at the opposite extremes for a polygenic trait. Their children will
    a. be bell-shaped.
    b. be a phenotype typical of a 9:3:3:1 ratio.
    c. have the middle phenotype between their two parents.
    d. look like one parent or the other.
19. Determine if the characteristic possessed by the shaded squares (males) and circles (females) is an autosomal dominant, autosomal recessive, or X-linked recessive.



additional genetics problems*

1. If a man homozygous for widow's peak (dominant) reproduces with a woman homozygous for straight hairline (recessive), what are the chances of their children having a widow's peak? A straight hairline?
2. A son with cystic fibrosis (autosomal recessive) is born to a couple who appear to be normal. What are the chances that any child born to this couple will have cystic fibrosis?
3. In horses, *B* = black coat and *b* = brown coat. What type of cross should be done to best determine whether a black-coated horse is homozygous dominant or heterozygous?
4. In a fruit fly experiment (see key on page 197), two gray-bodied fruit flies produce mostly gray-bodied offspring, but some offspring have black bodies. If there are 280 offspring, how many do you predict will have gray bodies and how many will have black bodies? How many of the 280 offspring do you predict will be heterozygous?
5. In humans, the allele for short fingers is dominant over that for long fingers. If a person with short fingers who had one parent with long fingers reproduces with a person having long fingers, what are the chances of each child having short fingers?
6. In humans, short fingers and widow's peak are dominant over long fingers and straight hairline. A heterozygote in both regards produces with a similar heterozygote. What is the chance of any one child having the same phenotype as the parents?
7. A man has type AB blood. What is his genotype? Could this man be the father of a child with type B blood? If so, what blood types could the child's mother have?
8. Is it possible for a woman who is homozygous dominant for normal color vision and a color-blind man to have a son who is color-blind? Why or why not?
9. Both the mother and father of a male hemophiliac appear normal. From whom did the son inherit the allele for hemophilia? What are the genotypes of the mother, the father, and the son?

*Answers to Additional Genetics Problems appear in Appendix A.

thinking scientifically

1. You want to determine whether a newly found *Drosophila* characteristic is dominant or recessive. Would you wait to cross this male fly with another of its own kind or cross it now with a fly that lacks the characteristic?
2. You want to test if the leaf pattern of a plant is influenced by the amount of fertilizer in the environment. What would you do?

Biology website

The companion website for *Biology* provides a wealth of information organized and integrated by chapter. You will find practice tests, animations, videos, and much more that will complement your learning and understanding of general biology.

http://www.mhhe.com/maderbiology10

12

Molecular Biology of the Gene

Nearly 1.5 million different species of organisms have been discovered and named. This number represents a small portion of the total number of species on Earth. It certainly represents a small fraction of the total number of species that have ever lived. Yet one gene differs from another only by the sequence of the nucleotide bases in DNA. How does a difference in base sequence determine the uniqueness of a species—for example, whether an individual is a daffodil or a gorilla? Or, for that matter, whether a human has blue, brown, or hazel eyes? By studying the activity of genes in cells, geneticists have confirmed that proteins are the link between the genotype and the phenotype. Mendel's peas are smooth or wrinkled according to the presence or absence of a starch-forming enzyme. The allele S *in peas dictates the presence of the starch-forming enzyme, whereas the allel* s *does not.*

Through its ability to specify proteins, DNA brings about the development of the unique structures that make up a particular type of organism. When studying gene expression in this chapter, keep in mind this flow diagram: DNA's sequence of nucleotides → sequences of amino acids → specific enzymes → structures in organism.

The diversity of life is dependent on gene activity.

concepts

12.1 The Genetic Material

The middle of the twentieth century was an exciting period of scientific discovery. On one hand, geneticists were busy determining that *DNA (deoxyribonucleic acid)* is the genetic material of living things. On the other hand, biochemists were in a frantic race to describe the structure of DNA. The classic experiments performed during this era set the stage for an explosion in our knowledge of modern molecular biology.

When researchers began their work, they knew that the genetic material must be

1. able to *store information* that pertains to the development, structure, and metabolic activities of the cell or organism;
2. stable so that it *can be replicated* with high fidelity during cell division and be transmitted from generation to generation;
3. able to *undergo rare changes* called mutations [L. *muta,* change] that provide the genetic variability required for evolution to occur.

This chapter will show, as the researchers of the twentieth century did, that DNA can fulfill these functions.

Transformation of Bacteria

During the late 1920s, the bacteriologist Frederick Griffith was attempting to develop a vaccine against *Streptococcus pneumoniae* (pneumococcus), which causes pneumonia in mammals. In 1931, he performed a classic experiment with the bacterium. He noticed that when these bacteria are grown on culture plates, some, called S strain bacteria, produce shiny, smooth colonies, and others, called R strain bacteria, produce colonies that have a rough appearance. Under the microscope, S strain bacteria have a capsule (mucous coat) but R strain bacteria do not. When Griffith injected mice with the S strain of bacteria, the mice died, and when he injected mice with the R strain, the mice did not die (Fig. 12.1). In an effort to determine if the capsule alone was responsible for the virulence (ability to kill) of the S strain bacteria, he injected mice with heat-killed S strain bacteria. The mice did not die.

Finally, Griffith injected the mice with a mixture of heat-killed S strain and live R strain bacteria. Most unexpectedly, the mice died and living S strain bacteria were recovered from the bodies! Griffith concluded that some substance necessary for the bacteria to produce a capsule and be virulent must have passed from the dead S strain bacteria to the living R strain bacteria so that the R strain bacteria were *transformed* (Fig. 12.1*d*). This change in the phenotype of the R strain bacteria must be due to a change in their genotype. Indeed, couldn't the transforming substance that passed from S strain to R strain be genetic material? Reasoning such as this prompted investigators at the time to begin looking for the transforming substance to determine the chemical nature of the genetic material.

DNA: The Transforming Substance

By the time the next group of investigators, led by Oswald Avery, began their work, it was known that the genes are on the chromosomes and that the chromosomes contain both proteins and nucleic acids. Investigators were having a much heated debate about whether protein or DNA was the genetic material. Many thought that the protein component of chromosomes must be the genetic material because proteins contain 20 different amino acids that can be sequenced in any particular way. On the other hand, nucleic acids—DNA and RNA—contain only four types of **nucleotides.** Perhaps DNA did not have enough variability to be able to store information and be the genetic material!

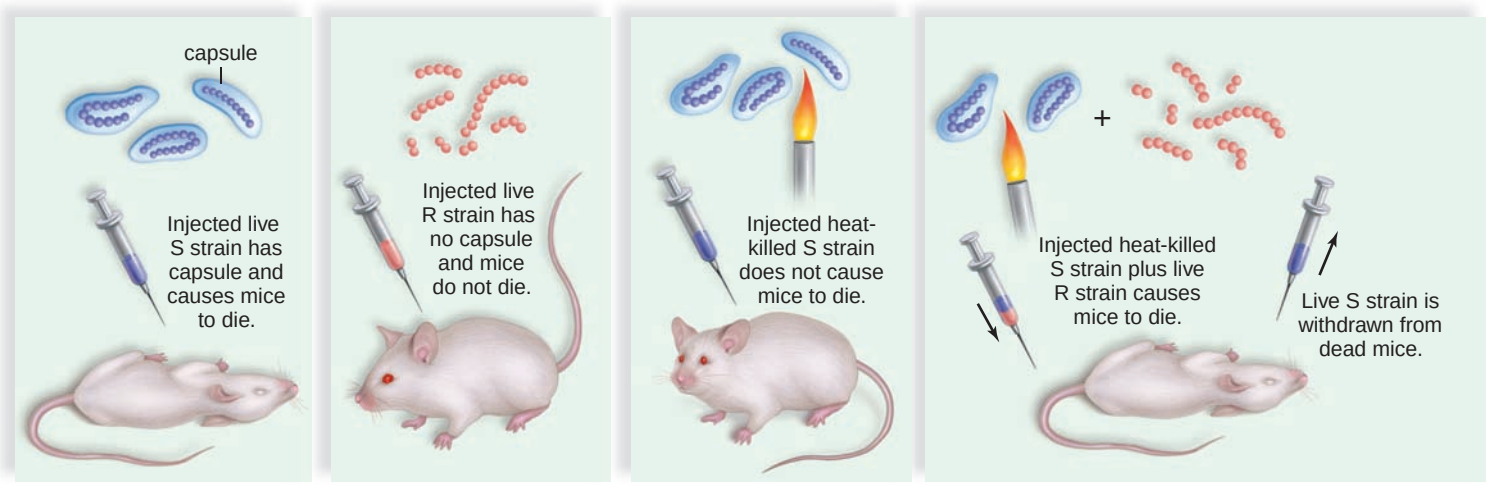


FIGURE 12.1 Griffith's transformation experiment.

a. Encapsulated S strain is virulent and kills mice. **b.** Nonencapsulated R strain is not virulent and does not kill mice. **c.** Heat-killed S strain bacteria do not kill mice. **d.** If heat-killed S strain and R strain are both injected into mice, they die because the R strain bacteria have been transformed into the virulent S strain.

Not surprising, Oswald Avery did not work with mice—it's inconvenient to be looking for the substance that transforms bacteria in mice. Avery and his group did in vitro experiments (in laboratory glassware). In 1944, after 16 years of research, Avery and his coinvestigators, Colin MacLeod and Maclyn McCarty, published a paper demonstrating that the transforming substance that allows *Streptococcus* to produce a capsule and be virulent is DNA. This meant that DNA is the genetic material. Their evidence included the following data:

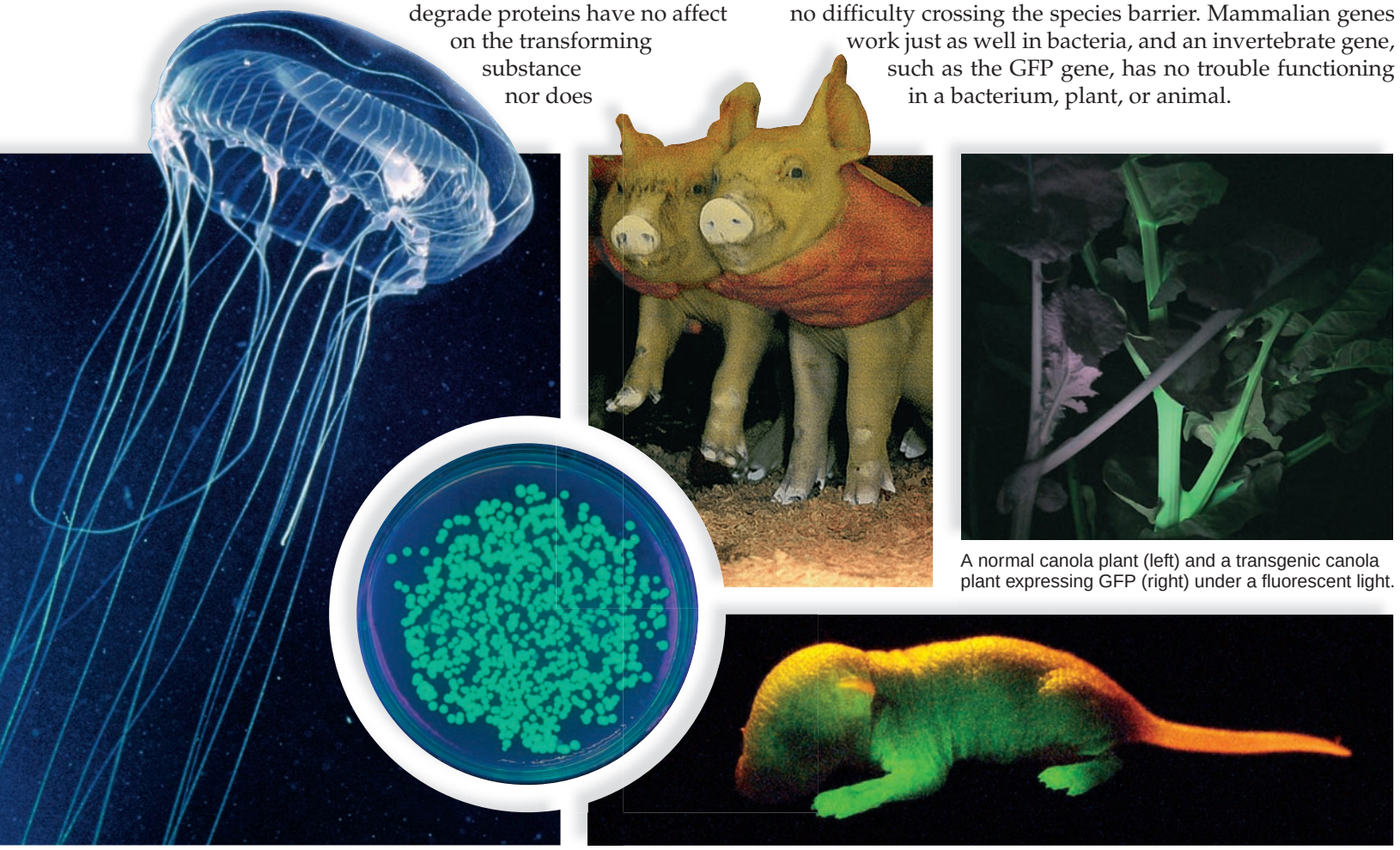
1. DNA from S strain bacteria causes R strain bacteria to be transformed so that they can produce a capsule and be virulent. We know today that DNA codes for the enzymes that allow bacteria to make a capsule.
2. The addition of DNase, an enzyme that digests DNA, prevents transformation from occurring. This supports the hypothesis that DNA is the genetic material.
3. The molecular weight of the transforming substance is so great that it must contain about 1,600 nucleotides! Certainly this suggests the possibility of genetic variability.
4. The addition of enzymes that degrade proteins have no affect on the transforming substance nor does RNase, an enzyme that digests RNA. This shows that neither protein nor RNA is the genetic material.

These experiments certainly showed that DNA is the transforming substance and, therefore, the genetic material. Although some remained skeptical, many felt that the evidence for DNA being the genetic material was overwhelming.

Transformation of Organisms Today

Transformation of organisms, resulting in so-called genetically modified organisms (GMOs), is an invaluable tool in modern biotechnology today. As discussed further in the next chapter, transformation of bacteria and other organisms has resulted in commercial products that are currently much used. Early biotechnologists seeking a dramatic way to show that it was possible to transfer a gene from one type of organism to another decided to make use of a jellyfish gene that codes for a green fluorescent protein (GFP).

When this gene is transferred to another organism, the organism glows in the dark (Fig. 12.2)! The basic technique is relatively simple. First, isolate the jellyfish gene and then transfer it to a bacterium, or the embryo of a plant, pig, or mouse. The result is a bioluminescent organism. Genes have no difficulty crossing the species barrier. Mammalian genes work just as well in bacteria, and an invertebrate gene, such as the GFP gene, has no trouble functioning in a bacterium, plant, or animal.



A normal canola plant (left) and a transgenic canola plant expressing GFP (right) under a fluorescent light.

FIGURE 12.2 Transformation of organisms.

When bacteria, plants, pigs, and mice are given a jellyfish gene for green fluorescent protein (GFP), these organisms glow in the dark.

The Structure of DNA

By the 1950s, DNA was widely accepted as the genetic material of living things. But another fundamental question remained—what exactly is the structure of DNA, and how can a molecule with only four different nucleotides produce the great diversity of life on Earth?

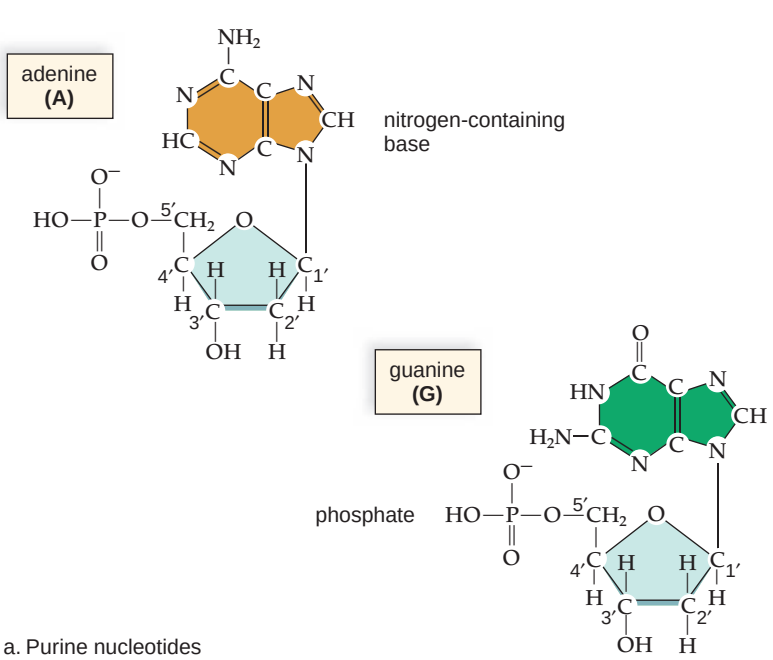
One obstacle in describing the structure of DNA is understanding the base composition of DNA. To accomplish this, it is possible to turn to the work of Erwin Chargaff, who used new chemical techniques developed in the 1940s to analyze in detail the base content of DNA. It was known that DNA contains four different types of nucleotides: two with *purine* bases, **adenine (A)** and **guanine (G),** which have a double ring, and two with *pyrimidine* bases, **thymine (T)** and **cytosine (C),** which have a single ring (Fig. 12.3*a, b*). At first, chemists hypothesized that DNA has repeating units, each unit having four nucleotides—one for each of the four bases. If so, the DNA of every species would contain 25% of each kind of nucleotide.

A sample of Chargaff's data is seen in Figure 12.3*c.* You can see that while some species—*E. coli* and *Zea mays* (corn), for example—do have approximately 25% of each type of nucleotide, most do not. Further, the percentage of each type of nucleotide differs from species to species. Therefore, the nucleotide content of DNA is not fixed, and DNA does have the *variability* between species required of the genetic material.

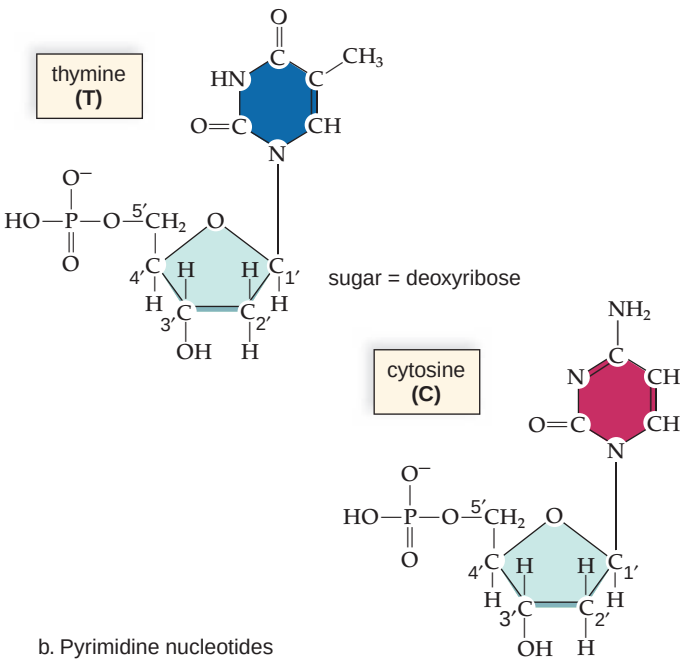
Within each species, however, DNA was found to have the *constancy* required of the genetic material—that is, all members of a species have the same base composition. Also, the percentage of A always equals the percentage of T, and the percentage of G equals the percentage of C. The percentage of A + G equals 50%, and the percentage of T + C equals 50%. These relationships are called Chargaff's rules.

Chargaff's rules:

1. The amount of A, T, G, and C in DNA varies from species to species.
2. In each species, the amount of A = T and the amount of G = C.



a. Purine nucleotides



b. Pyrimidine nucleotides

DNA Composition in Various Species (%)				
Species	**A**	**T**	**G**	**C**
Homo sapiens (human)	31.0	31.5	19.1	18.4
Drosophila melanogaster (fruit fly)	27.3	27.6	22.5	22.5
Zea mays (corn)	25.6	25.3	24.5	24.6
Neurospora crassa (fungus)	23.0	23.3	27.1	26.6
Escherichia coli (bacterium)	24.6	24.3	25.5	25.6
Bacillus subtilis (bacterium)	28.4	29.0	21.0	21.6

c. Chargaff's data

FIGURE 12.3 Nucleotide composition of DNA.

All nucleotides contain phosphate, a 5-carbon sugar, and a nitrogen-containing base. In DNA, the sugar is called deoxyribose because it lacks an oxygen atom in the 2′ position, compared to ribose. The nitrogen-containing bases are (**a**) the purines adenine and guanine, which have a double ring, and (**b**) the pyrimidines thymine and cytosine, which have a single ring. **c.** Chargaff's data show that the DNA of various species differs. For example, in humans the A and T percentages are about 31%, but in fruit flies these percentages are about 27%.

While there are only four possible bases in each nucleotide position in DNA, the sheer length of most DNA molecules is more than sufficient to provide for variability. For example, it has been calculated that each human chromosome usually contains about 140 million base pairs. This provides for a staggering number of possible sequences of nucleotides. Because any of the four possible nucleotides can be present at each nucleotide position, the total number of possible nucleotide sequences is $4^{140 \times 10^6}$ or $4^{140,000,000}$. No wonder each species has its own base percentages!

X-Ray Diffraction of DNA

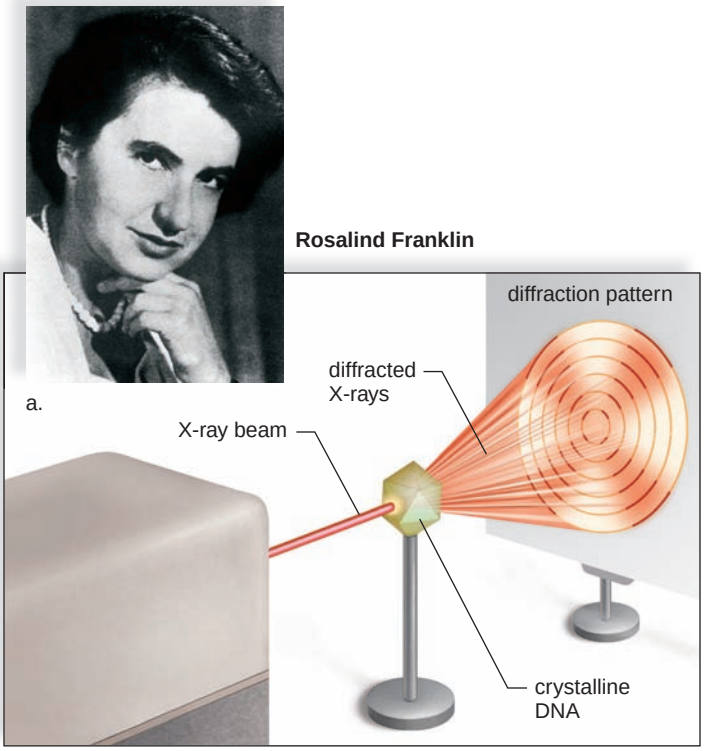
Rosalind Franklin (Fig. 12.4*a*), a researcher in the laboratory of Maurice H. F. Wilkins at King's College in London, studied the structure of DNA using X-rays. She found that if a concentrated, viscous solution of DNA is made, it can be separated into fibers. Under the right conditions, the fibers are enough like a crystal (a solid substance whose atoms are arranged in a definite manner) that when X-rayed, an X-ray diffraction pattern results (Fig. 12.4*b*). The X-ray diffraction pattern of DNA shows that DNA is a double helix. The helical shape is indicated by the crossed (X) pattern in the center of the photograph in Figure 12.4*c*. The dark portions at the top and bottom of the photograph indicate that some portion of the helix is repeated.

The Watson and Crick Model

James Watson, an American, was on a postdoctoral fellowship at Cavendish Laboratories in Cambridge, England, and while there he began to work with the biophysicist Francis H. C. Crick. Using the data provided from X-ray diffraction and other sources, they constructed a model of DNA for which they received a Nobel Prize in 1962.

Watson and Crick knew, of course, that DNA is a polymer of nucleotides, but they did not know how the nucleotides were arranged within the molecule. However, they deduced that DNA is a **double helix** with sugar-phosphate backbones on the outside and paired bases on the inside. This arrangement fits the mathematical measurements provided by Franklin's X-ray diffraction data for the spacing between the base pairs (0.34 nm) and for a complete turn of the double helix (3.4 nm).

According to Watson and Crick's model, the two DNA strands of the double helix are antiparallel, meaning that the sugar-phosphate groups of each strand are oriented in opposite directions. This means that the 5′ end of one strand is paired to the 3′ end of the other strand, and vice versa.



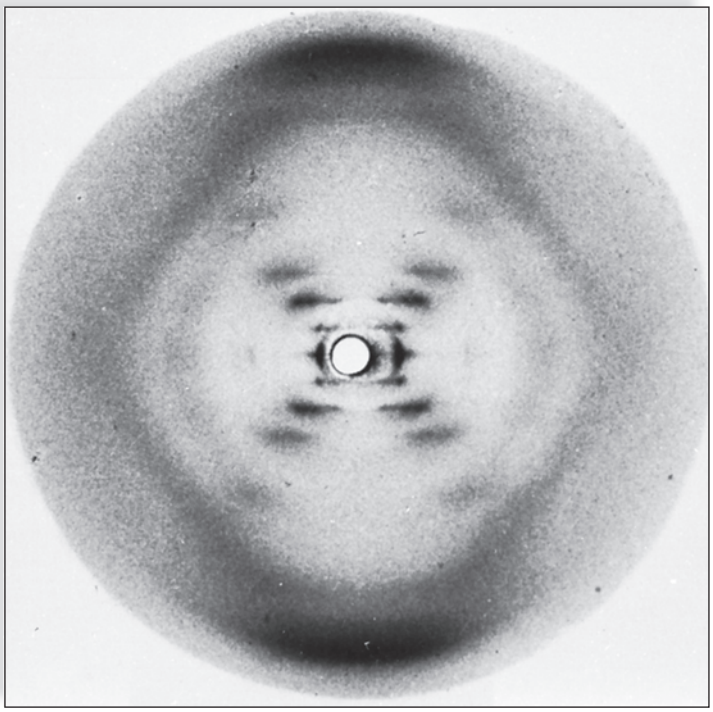


FIGURE 12.4 X-ray diffraction of DNA.

a. Rosalind Franklin, 1920–1958. **b.** When a crystal is X-rayed, the way in which the beam is diffracted reflects the pattern of the molecules in the crystal. The closer together two repeating structures are in the crystal, the farther from the center the beam is diffracted. **c.** The diffraction pattern of DNA produced by Rosalind Franklin. The crossed (X) pattern in the center told investigators that DNA is a helix, and the dark portions at the top and the bottom told them that some feature is repeated over and over. Watson and Crick determined that this feature was the hydrogen-bonded bases.

This model also agreed with Chargaff's rules, which said that A = T and G = C. Figure 12.5 shows that A is hydrogen-bonded to T, and G is hydrogen-bonded to C. This so-called **complementary base pairing** means that a purine is always bonded to a pyrimidine. The antiparallel arrangement of the two strands ensures that the bases are oriented properly so that they can interact. Only in this way will the molecule have the width revealed by Franklin's X-ray diffraction pattern, since two pyrimidines together are too narrow, and two purines together are too wide (Fig. 12.5).

The information stored within DNA must always be read in the correct order. As explained on page 218, each nucleotide possesses a phosphate group located at the 5′ position of the sugar. Nucleotides are joined together by linking the 5′ phosphate of one nucleotide to a free hydroxyl (—OH) located at the 3′ position on the sugar of the preceding nucleotide, giving the molecule directionality. Thus, a DNA strand is usually made in a 5′ to 3′ direction.

Check Your Progress 12.1

1. What are the requirements for DNA to be the genetic material?
2. What are the major features of DNA structure?

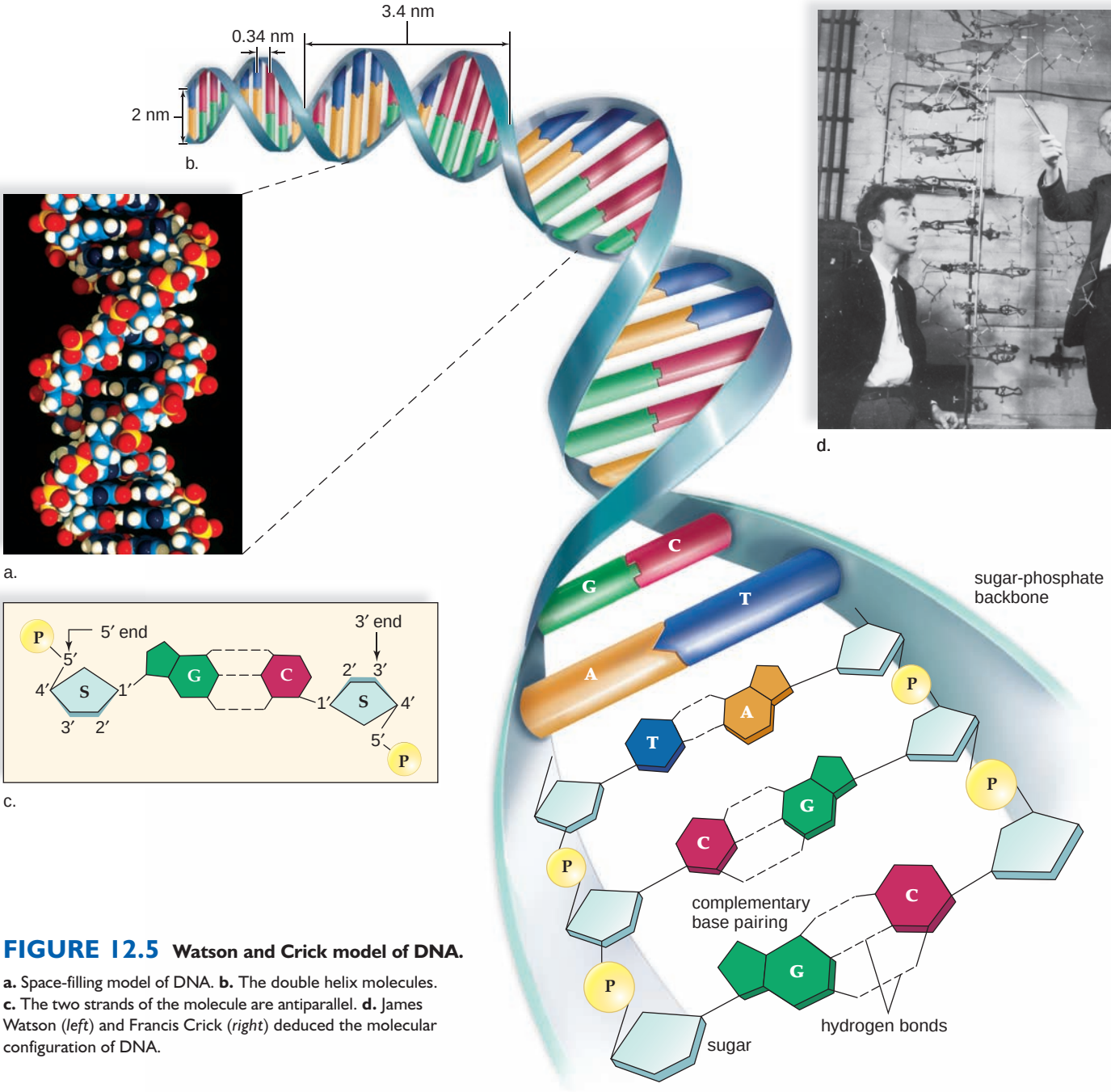


FIGURE 12.5 Watson and Crick model of DNA.

a. Space-filling model of DNA. **b.** The double helix molecules. **c.** The two strands of the molecule are antiparallel. **d.** James Watson (*left*) and Francis Crick (*right*) deduced the molecular configuration of DNA.

12.2 Replication of DNA

The term **DNA replication** refers to the process of copying a DNA molecule. Following replication, there is usually an exact copy of the parental DNA double helix. As soon as Watson and Crick developed their double-helix model, they commented, "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

A **template** is most often a mold used to produce a shape complementary to itself. During DNA replication, each DNA strand of the parental double helix serves as a template for a new strand in a daughter molecule (Fig. 12.6). DNA replication is termed **semiconservative replication** because each daughter DNA double helix contains an old strand from the parental DNA double helix and a new strand.

Replication requires the following steps:

1. *Unwinding.* The old strands that make up the parental DNA molecule are unwound and "unzipped" (i.e., the weak hydrogen bonds between the paired bases are broken). A special enzyme called helicase unwinds the molecule.
2. *Complementary base pairing.* New complementary nucleotides, always present in the nucleus, are positioned by the process of complementary base pairing.
3. *Joining.* The complementary nucleotides join to form new strands. Each daughter DNA molecule contains an old strand and a new strand.

Steps 2 and 3 are carried out by an enzyme complex called **DNA polymerase.**[1] DNA polymerase works in the test tube as well as in cells.

In Figure 12.6, the backbones of the parental DNA molecule are bluish, and each base is given a particular color. Following replication, the daughter molecules each have a greenish backbone (new strand) and a bluish backbone (old strand). A daughter DNA double helix has the same sequence of bases as the parental DNA double helix had originally. Although DNA replication can be explained easily in this manner, it is actually a complicated process. Some of the more precise molecular events are discussed in the Science Focus reading on page 218.

DNA replication must occur before a cell can divide. Cancer, which is characterized by rapidly dividing cells, is sometimes treated with chemotherapeutic drugs that are analogs (have a similar, but not identical, structure) to one of the four nucleotides in DNA. When these are mistakenly used by the cancer cells to synthesize DNA, replication stops and the cells die off.

[1] The complex contains a number of different DNA polymerases with specific functions.

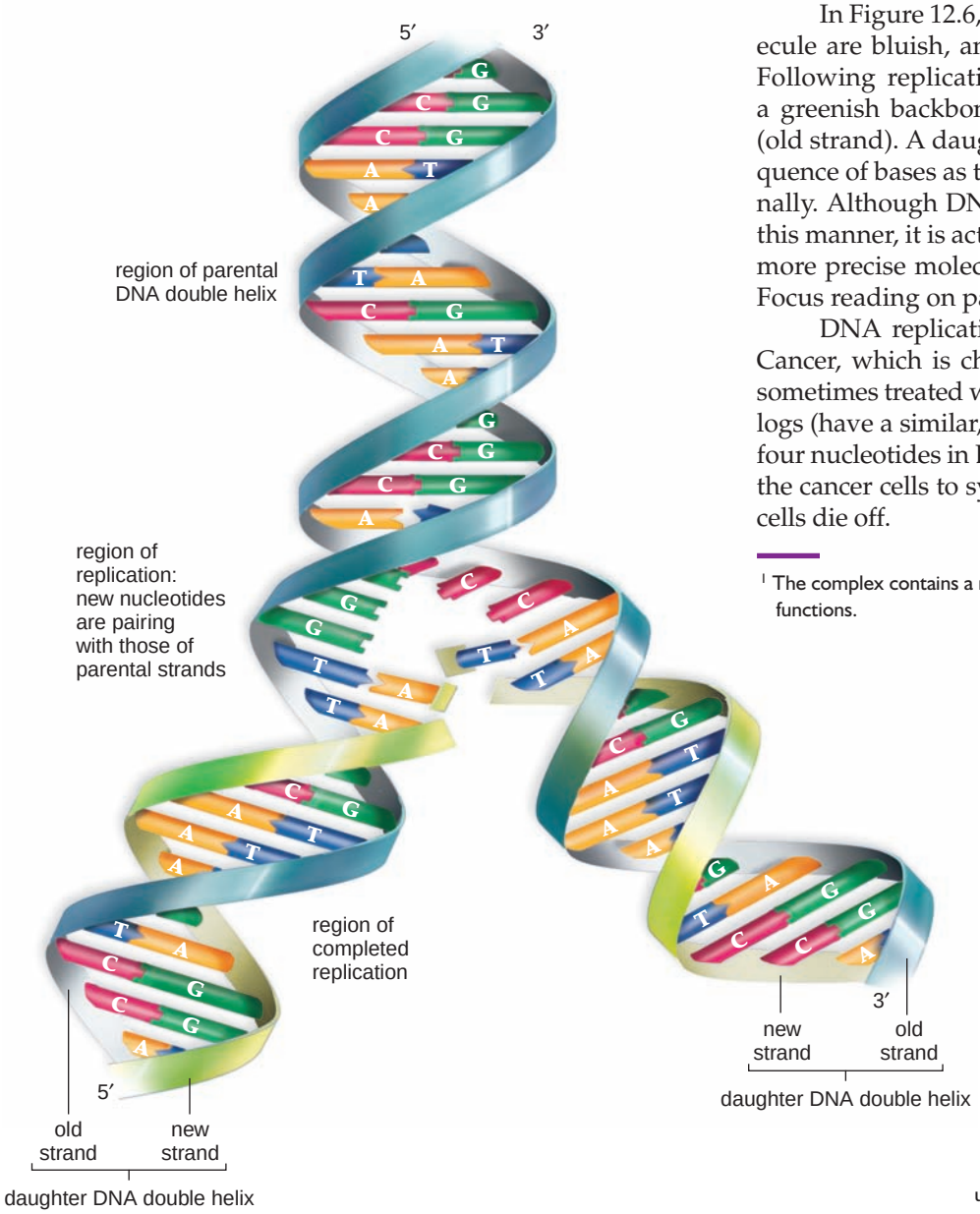
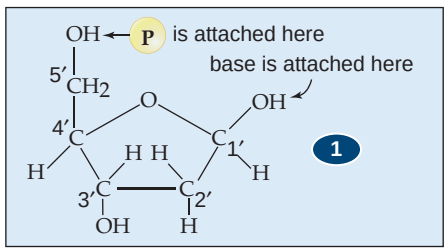


FIGURE 12.6 Semiconservative replication (simplified).

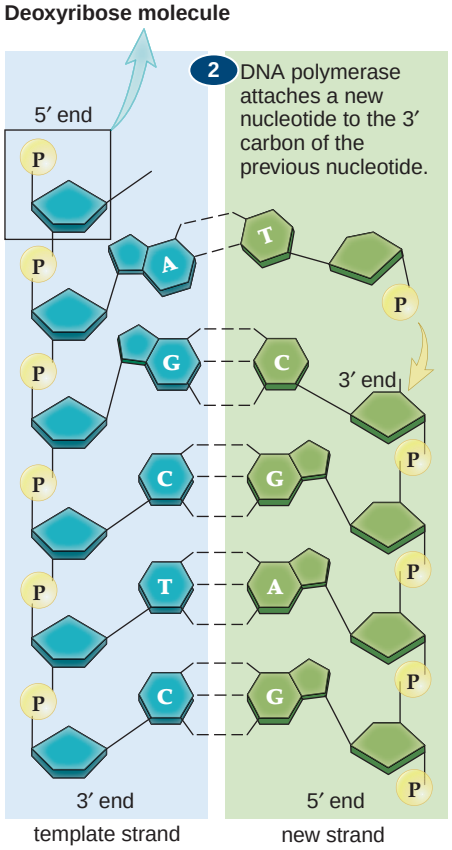
After the DNA double helix unwinds, each old strand serves as a template for the formation of the new strand. Complementary nucleotides available in the cell pair with those of the old strand and then are joined together to form a strand. After replication is complete, there are two daughter DNA double helices. Each is composed of an old strand and a new strand. Each daughter double helix has the same sequence of base pairs as the parental double helix had before unwinding occurred.

science focus

Aspects of DNA Replication



Deoxyribose molecule



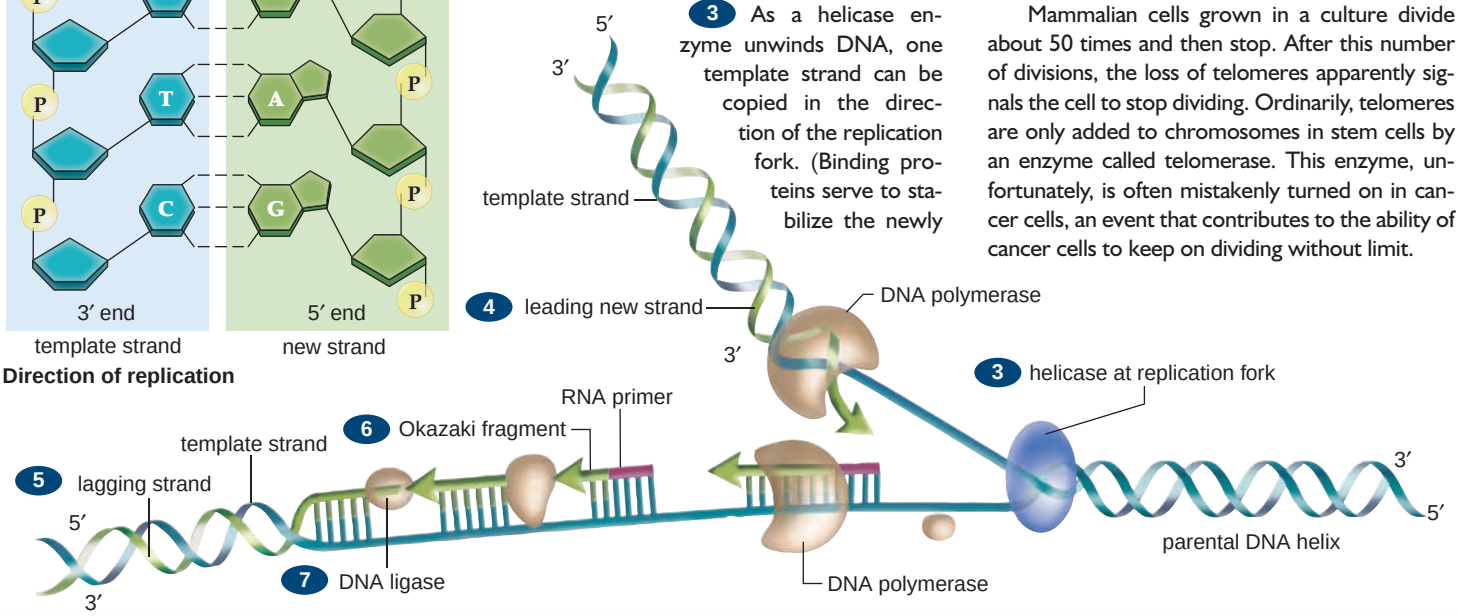
Direction of replication

Watson and Crick realized that the strands in DNA had to be antiparallel to allow for complementary base pairing. This opposite polarity of the strands introduces complications for DNA replication, as we will now see. In Figure 12A, 1 take a look at a deoxyribose molecule, in which the carbon atoms are numbered. Use the structure to see that 2 the DNA strand in the blue box runs opposite from the DNA strand in the green box. In other words, the strand in the blue box has a 5′ end at the top, and the strand in the green box has a 3′ end at the top. During replication, DNA polymerase has to join the nucleotides of the new strand so that the 3′ end is uppermost. Why? Because DNA polymerase can only join a nucleotide to the free 3′ end of the previous nucleotide, as shown. Also, *DNA polymerase cannot start the synthesis of a DNA chain.* Therefore, an RNA polymerase (see page 222) lays down a short amount of RNA, called an RNA primer, that is complementary to the template strand being replicated. After that, DNA polymerase can join DNA nucleotides to the 3′ end of the growing new strand.

3 As a helicase enzyme unwinds DNA, one template strand can be copied in the direction of the replication fork. (Binding proteins serve to stabilize the newly formed, single-stranded regions.) 4 This strand is called the leading new strand. The other template strand has to be copied in the direction away from the fork. Therefore, replication must begin over and over again as the DNA molecule unwinds. 5 Replication of this so-called lagging new strand is, therefore, discontinuous, and it results in segments called 6 Okazaki fragments, after the Japanese scientist Reiji Okazaki, who discovered them.

Replication is only complete when the RNA primers are removed. This works out well for the lagging new strand. While proofreading, DNA polymerase removes the RNA primers and replaces them with complementary DNA nucleotides. 7 Another enzyme, called DNA ligase, joins the fragments. However, there is no way for DNA polymerase to replicate the 5′ ends of both new strands after RNA primers are removed. This means that DNA molecules get shorter as one replication follows another. The ends of eukaryotic DNA molecules have a special nucleotide sequence called a telomere. **Telomeres** do not code for proteins and, instead, are repeats of a short nucleotide sequence, such as TTAGGG.

Mammalian cells grown in a culture divide about 50 times and then stop. After this number of divisions, the loss of telomeres apparently signals the cell to stop dividing. Ordinarily, telomeres are only added to chromosomes in stem cells by an enzyme called telomerase. This enzyme, unfortunately, is often mistakenly turned on in cancer cells, an event that contributes to the ability of cancer cells to keep on dividing without limit.



Replication fork introduces complications

FIGURE 12A DNA replication (in depth).

Prokaryotic Versus Eukaryotic Replication

The process of DNA replication is distinctly different in prokaryotic and eukaryotic cells (Fig. 12.7).

Prokaryotic DNA Replication

Bacteria have a single circular loop of DNA that must be replicated before the cell divides. In some circular DNA molecules, replication moves around the DNA molecule in one direction only. In others, as shown in Figure 12.7*a*, replication occurs in two directions. The process always occurs in the 5′ to 3′ direction.

The process begins at the *origin of replication,* a specific site on the bacterial chromosome. The strands are separated and unwound, and a DNA polymerase binds to each side of the opening and begins the copying process. When the two DNA polymerases meet at a termination region, replication is halted, and the two copies of the chromosome are separated.

Bacterial cells require about 40 minutes to replicate the complete chromosome. Because bacterial cells are able to divide as often as once every 20 minutes, it is possible for a new round of DNA replication to begin even before the previous round is completed!

Eukaryotic DNA Replication

In eukaryotes, DNA replication begins at numerous origins of replication along the length of the chromosome, and the so-called replication bubbles spread bidirectionally until they meet. Notice in Figure 12.7*b* that there is a V shape wherever DNA is being replicated. This is called a **replication fork.**

The chromosomes of eukaryotes are long and linear, making replication a more time-consuming process. Eukaryotes replicate their DNA at a slower rate—500–5,000 base pairs per minute—but there are many individual origins of replication to accelerate the process. Therefore, eukaryotic cells complete the replication of the diploid amount of DNA (in humans, over 6 billion base pairs) in a matter of hours!

The linear chromosomes of eukaryotes also pose another problem—DNA polymerase is unable to replicate the ends of the chromosomes. The ends of eukaryotic chromosomes are composed of telomeres, which are short DNA sequences that are repeated over and over. Telomeres are not copied by DNA polymerase; rather, they are added by an enzyme called telomerase, which adds the repeats after the chromosome is replicated. In stem cells, this process preserves the ends of the chromosomes and prevents the loss of DNA after successive rounds of replication.

Accuracy of Replication

A DNA polymerase is very accurate and makes a mistake approximately once per 100,000 base pairs at most. This error rate, however, would result in many errors accumulating over the course of several cell divisions. DNA polymerase is also capable of proofreading the daughter strand it is making. It can recognize a mismatched nucleotide and remove it from a daughter strand by reversing direction and removing several nucleotides. Once it has removed the mismatched nucleotide, it changes direction again and resumes making DNA. Overall, the error rate for the bacterial DNA polymerase is only one in 100 million base pairs!

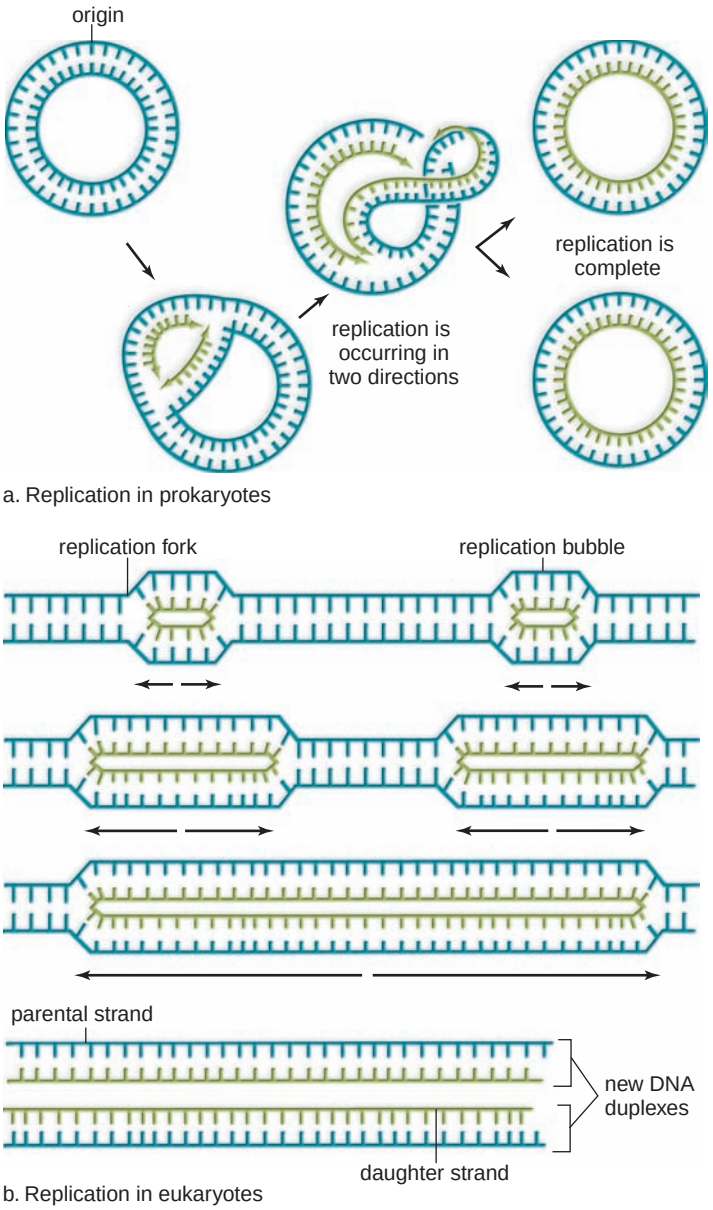


FIGURE 12.7 Prokaryotic versus eukaryotic replication.

a. In prokaryotes, replication can occur in two directions at once because the DNA molecule is circular. **b.** In eukaryotes, replication occurs at numerous replication forks. The bubbles thereby created spread out until they meet.

Check Your Progress 12.2

1. Describe the three major steps in DNA replication.
2. Why is DNA replication referred to as semiconservative?
3. How does DNA replication in eukaryotes differ from prokaryotic DNA replication?

12.3 The Genetic Code of Life

Evidence began to mount in the 1900s that metabolic disorders can be inherited. An English physician, Sir Archibald Garrod, called them "inborn errors of metabolism." Investigators George Beadle and Edward Tatum, working with red bread mold, discovered what they called the "one gene, one enzyme hypothesis," based on the observation that a defective gene caused a defective enzyme.

Other investigators decided to see if the protein hemoglobin in persons with the inherited condition sickle-cell disease (see page 203) has a structure different from normal hemoglobin. They found that the amino acid glutamate had been replaced by the amino acid valine in one location. This causes sickle cell hemoglobin to stack up into long, semi-rigid rods distorting red blood cells into the sickle shape.

These examples support the hypothesis that DNA, the genetic material, specifies proteins. It has been shown many times over by now that DNA specifies proteins with the help of RNA molecules.

TABLE 12.1

RNA Structure Compared to DNA Structure

	RNA	*DNA*
Sugar	Ribose	Deoxyribose
Bases	Adenine, guanine, uracil, cytosine	Adenine, guanine, thymine, cytosine
Strands	Single stranded	Double stranded with base pairing
Helix	No	Yes

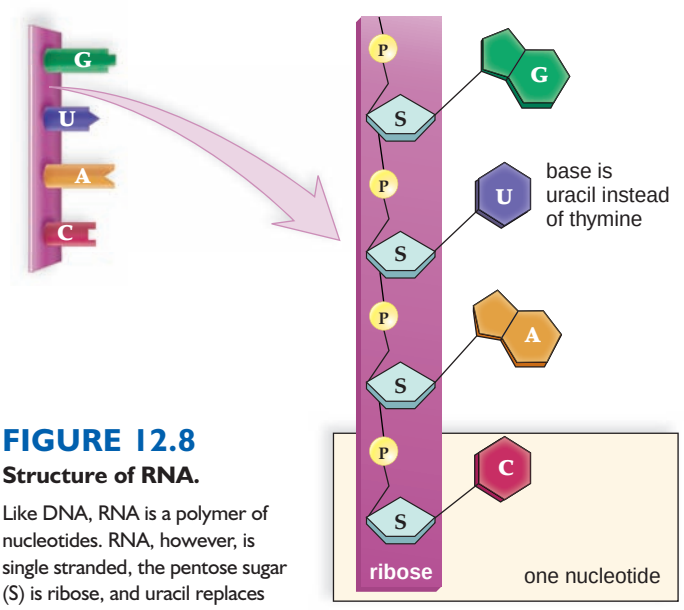


FIGURE 12.8
Structure of RNA.

Like DNA, RNA is a polymer of nucleotides. RNA, however, is single stranded, the pentose sugar (S) is ribose, and uracil replaces thymine as one of the bases.

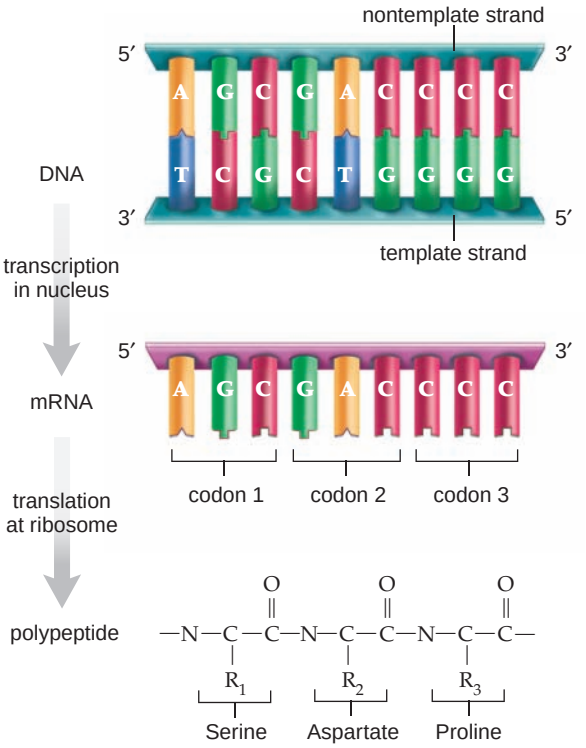


FIGURE 12.9 **The central dogma of molecular biology.**

One strand of DNA acts as a template for mRNA synthesis, and the sequence of bases in mRNA determines the sequence of amino acids in a polypeptide.

RNA Carries the Information

Like DNA, *RNA (ribonucleic acid)* is a polymer composed of nucleotides. The nucleotides in RNA, however, contain the sugar ribose and the bases adenine (A), cytosine (C), guanine (G), and **uracil (U).** In RNA, the base uracil replaces the thymine found in DNA. Finally, RNA is single stranded and does not form a double helix in the same manner as DNA (Table 12.1 and Fig. 12.8).

There are three major classes of RNA. Each class of RNA has its own unique size, shape, and function in protein synthesis.

Messenger RNA (mRNA) takes a message from DNA in the nucleus to the ribosomes in the cytoplasm.

Transfer RNA (tRNA) transfers amino acids to the ribosomes.

Ribosomal RNA (rRNA), along with ribosomal proteins, makes up the ribosomes, where polypeptides are synthesized.

The Genetic Code

There are two major steps in synthesizing a protein based on the information stored in DNA (Fig. 12.9). First, during **transcription** [L. *trans*, across, and *scriptio*, a writing], DNA serves as a template for RNA formation. DNA is transcribed monomer by monomer into another type of polynucleotide (RNA). Second, during **translation** [L. *trans*, across, and *latus*, carry or bear], the mRNA transcript directs the sequence of amino acids in a poly-

peptide. Like a translator who understands two languages, the cell changes a nucleotide sequence into an amino acid sequence. With the help of the three types of RNA, a gene (a segment of DNA) specifies the sequence of amino acids in a polypeptide. Together, the flow of information from DNA to protein is known as the central dogma of molecular biology.

Therefore, it is obvious that the sequence of nucleotides in DNA and mRNA specify the order of amino acids in a polypeptide. It would seem then that there must be a **genetic code** for each of the 20 amino acids found in proteins. But can four nucleotides provide enough combinations to code for 20 amino acids? If each code word, called a **codon,** were made up of two bases, such as AG, there could be only 16 codons. But if each codon were made up of three bases, such as AGC, there would be 64 codons—more than enough to code for 20 amino acids:

number of bases in genetic code	number of different amino acids specified
1	4
2	16
3	64

The genetic code is a **triplet code.** Each codon consists of three nucleotide bases, such as AUC.

Finding the Genetic Code

In 1961, Marshall Nirenberg and J. Heinrich Matthei performed an experiment that laid the groundwork for cracking the genetic code. First, they found that a cellular enzyme could be used to construct a synthetic RNA (one that does not occur in cells), and then they found that the synthetic RNA polymer could be translated in a test tube that contains the cytoplasmic contents of a cell. Their first synthetic RNA was composed only of uracil, and the protein that resulted was composed only of the amino acid phenylalanine. Therefore, the mRNA codon for phenylalanine was known to be UUU. Later, they were able to translate just three nucleotides at a time; in that way, it was possible to assign an amino acid to each of the mRNA codons (Fig. 12.10).

A number of important properties of the genetic code can be seen by careful inspection of Figure 12.10.

1. The genetic code is degenerate. This means that most amino acids have more than one codon; leucine, serine, and arginine have six different codons, for example. The degeneracy of the code protects against potentially harmful effects of mutations.
2. The genetic code is unambiguous. Each triplet codon has only one meaning.
3. The code has start and stop signals. There is only one start signal, but there are three stop signals.

The Code Is Universal

With a few exceptions, the genetic code (Fig. 12.10) is universal to all living things. In 1979, however, researchers discovered that the genetic code used by mammalian mitochondria and chloroplasts differs slightly from the more familiar genetic code.

First Base	Second Base U	Second Base C	Second Base A	Second Base G	Third Base
U	UUU phenylalanine	UCU serine	UAU tyrosine	UGU cysteine	U
	UUC phenylalanine	UCC serine	UAC tyrosine	UGC cysteine	C
	UUA leucine	UCA serine	UAA *stop*	UGA *stop*	A
	UUG leucine	UCG serine	UAG *stop*	UGG tryptophan	G
C	CUU leucine	CCU proline	CAU histidine	CGU arginine	U
	CUC leucine	CCC proline	CAC histidine	CGC arginine	C
	CUA leucine	CCA proline	CAA glutamine	CGA arginine	A
	CUG leucine	CCG proline	CAG glutamine	CGG arginine	G
A	AUU isoleucine	ACU threonine	AAU asparagine	AGU serine	U
	AUC isoleucine	ACC threonine	AAC asparagine	AGC serine	C
	AUA isoleucine	ACA threonine	AAA lysine	AGA arginine	A
	AUG *(start)* methionine	ACG threonine	AAG lysine	AGG arginine	G
G	GUU valine	GCU alanine	GAU aspartate	GGU glycine	U
	GUC valine	GCC alanine	GAC aspartate	GGC glycine	C
	GUA valine	GCA alanine	GAA glutamate	GGA glycine	A
	GUG valine	GCG alanine	GAG glutamate	GGG glycine	G

FIGURE 12.10 Messenger RNA codons.

Notice that in this chart, each of the codons (in boxes) is composed of three letters representing the first base, second base, and third base. For example, find the box where C for the first base and A for the second base intersect. You will see that U, C, A, or G can be the third base. The bases CAU and CAC are codons for histidine; the bases CAA and CAG are codons for glutamine.

The universal nature of the genetic code provides strong evidence that all living things share a common evolutionary heritage. Since the same genetic code is used by all living things, it is possible to transfer genes from one organism to another. Many commercial and medicinal products such as insulin can be produced in this manner. Genetic engineering has also produced some unusual organisms such as mice that literally glow in the dark. In this case, the gene responsible for bioluminescence in jellyfish was placed into mouse embryos (see Fig. 12.2).

Check Your Progress 12.3

1. What are the three major classes of RNA, and what are their functions?
2. What does it mean to say that the genetic code is degenerate?

12.4 First Step: Transcription

During *transcription,* a segment of the DNA serves as a template for the production of an RNA molecule. Although all three classes of RNA are formed by transcription, we will focus right now on transcription to form mRNA, the type of RNA that eventually leads to building a polypeptide as a gene product.

Messenger RNA Is Formed

An mRNA molecule has a sequence of bases complementary to a portion of one DNA strand; wherever A, T, G, or C is present in the DNA template, U, A, C, or G, respectively, is incorporated into the mRNA molecule (Fig. 12.11). When a gene is transcribed, a segment of the DNA helix unwinds and unzips, and complementary RNA nucleotides pair with DNA nucleotides of the strand opposite the gene. This strand is known as the *template strand*; the other strand is the nontemplate strand. An RNA polymerase joins the nucleotides together in the 5′ ⟶ 3′ direction. In other words, an **RNA polymerase** only adds a nucleotide to the 3′ end of the polymer under construction.

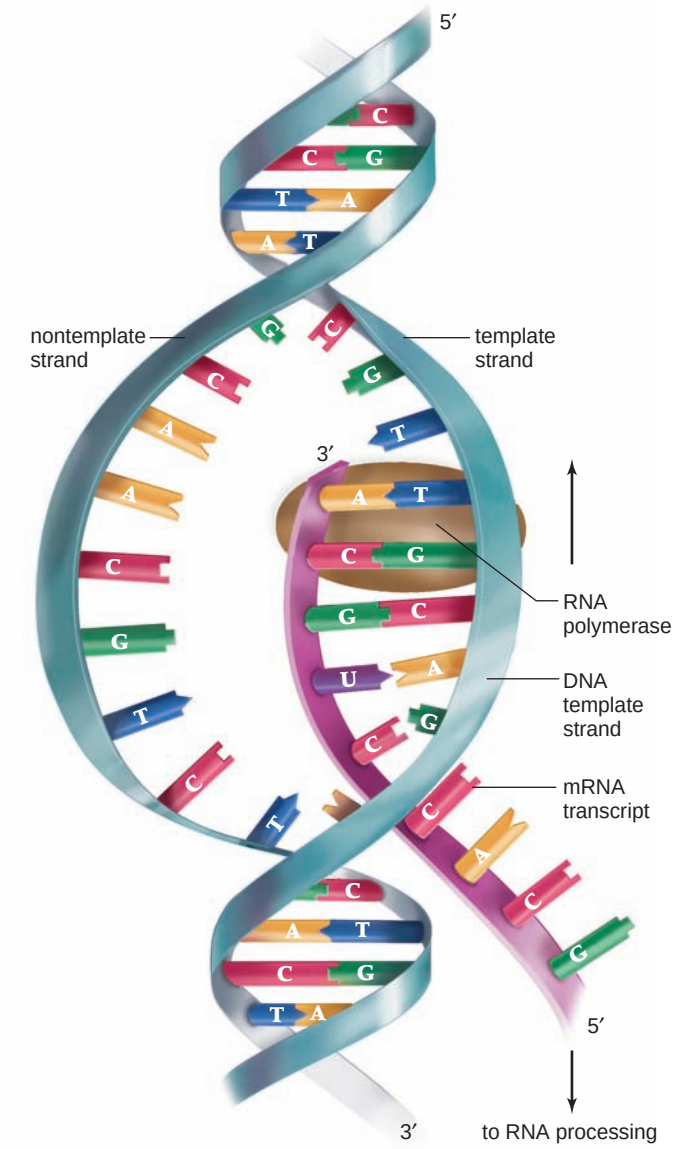
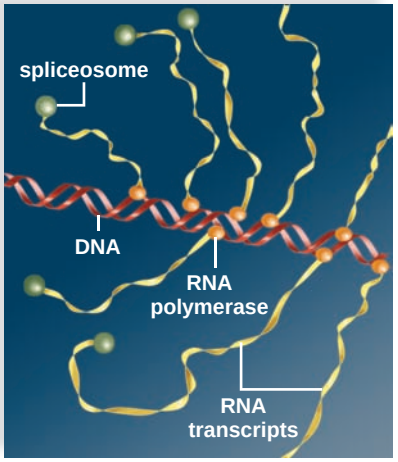


FIGURE 12.11 Transcription.

During transcription, complementary RNA is made from a DNA template. At the point of attachment of RNA polymerase, the DNA helix unwinds and unzips, and complementary RNA nucleotides are joined together. After RNA polymerase has passed by, the DNA strands rejoin and the mRNA transcript dangles to the side.

a.



b.

FIGURE 12.12
RNA polymerase.

a. Numerous RNA transcripts extend from a horizontal gene in an amphibian egg cell. **b.** The strands get progressively longer because transcription begins to the left. The dots along the DNA are RNA polymerase molecules. The dots at the end of the strands are spliceosomes involved in RNA processing (see Fig. 12.13).

Transcription begins when RNA polymerase attaches to a region of DNA called a promoter. A **promoter** defines the start of transcription, the direction of transcription, and the strand to be transcribed. The binding of RNA polymerase to the promoter is the *initiation* of transcription. The RNA-DNA association is not as stable as the DNA helix. Therefore, only the newest portion of an RNA molecule that is associated with RNA polymerase is bound to the DNA, and the rest dangles off to the side. *Elongation* of the mRNA molecule continues until RNA polymerase comes to a DNA stop sequence. The stop sequence causes RNA polymerase to stop transcribing the DNA and to release the mRNA molecule, now called an **mRNA transcript.**

Many RNA polymerase molecules can be working to produce mRNA transcripts at the same time (Fig. 12.12). This allows the cell to produce many thousands of copies of the same mRNA molecule, and eventually many copies of the same protein, within a shorter period of time than if the single copy of DNA were used to direct protein synthesis.

It is of interest that either strand of DNA can be a template strand. In other words, each strand of DNA can be a template strand but for a different gene.

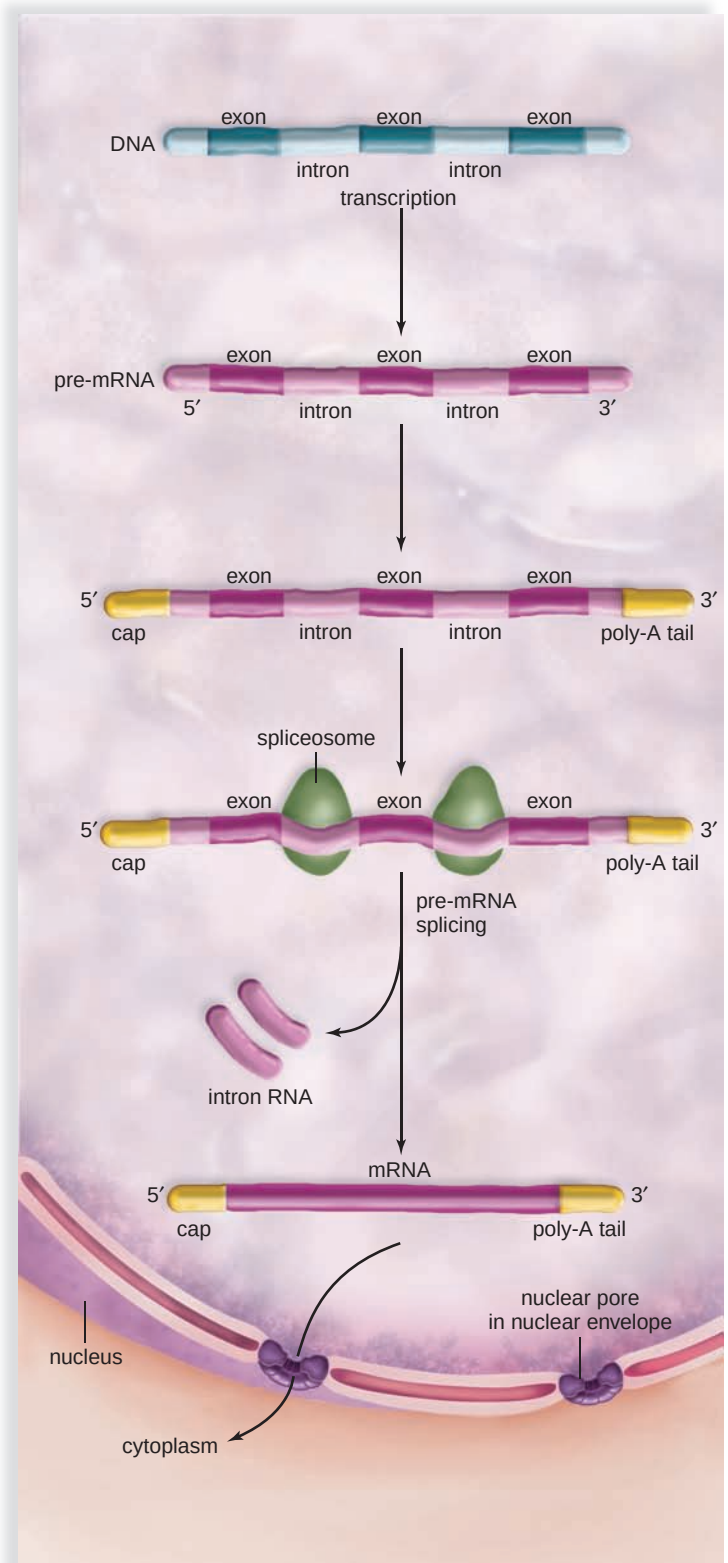


FIGURE 12.13 Messenger RNA (mRNA) processing in eukaryotes.

DNA contains both exons (protein-coding sequences) and introns (non-protein-coding sequences). Both of these are transcribed and are present in pre-mRNA. During processing, a cap and a poly-A tail (a series of adenine nucleotides) are added to the molecule. Also, there is excision of the introns and a splicing together of the exons. This is accomplished by complexes called spliceosomes. Then the mRNA molecule is ready to leave the nucleus.

RNA Molecules Are Processed

A newly formed RNA transcript, called a pre-mRNA, is modified before leaving the eukaryotic nucleus. For example, the molecule receives a cap at the 5′ end and a tail at the 3′ end (Fig. 12.13). The *cap* is a modified guanine (G) nucleotide that helps tell a ribosome where to attach when translation begins. The tail consists of a chain of 150–200 adenine (A) nucleotides. This so-called *poly-A tail* facilitates the transport of mRNA out of the nucleus and also inhibits degradation of mRNA by hydrolytic enzymes.

Also, the pre-mRNA, particularly in multicellular eukaryotes, is composed of exons and introns. The exons of the pre-mRNA molecule will be *ex*pressed, but not the **introns,** which occur *in* between the **exons.** During pre-mRNA splicing, the introns are removed. In prokaryotes, introns are removed by "self-splicing"—that is, the intron itself has the capability of enzymatically splicing itself out of a pre-mRNA. In eukaryotes, the RNA splicing is done by spliceosomes, which contain *small nuclear RNAs (snRNAs).* By means of complementary base pairing, snRNAs are capable of identifying the introns to be removed. A spliceosome utilizes a ribozyme when it removes the introns. **Ribozymes,** also found in prokaryotes, are RNA molecules that possess catalytic activity in the same manner as enzymes composed of protein. Following splicing, an mRNA is ready to leave the nucleus.

Another type of RNA called small nucleolar RNA (snoRNA) is present in the nucleolus, where it helps process rRNA and tRNA molecules.

Function of Introns

The presence of introns allows a cell to pick and choose which exons will go into a particular mRNA (see pages 242–43). That is, it has been discovered that an mRNA can contain only some of the possible exons available from a DNA sequence. Therefore, what is an exon in one mRNA could be an intron in another mRNA. This is called *alternative mRNA splicing*. Because the snRNAs play a role in determining what is an exon or intron for a particular mRNA, they take on greater significance in eukaryotes. Some introns give rise to *microRNAs (miRNAs),* which are involved in regulating the translation of mRNAs. These molecules bond with the mRNA through complementary base pairing and, in that way, prevent translation from occurring.

It is also possible that the presence of introns encourages crossing-over during meiosis, and this permits so-called *exon shuffling,* which can play a role in the evolution of new genes.

Check Your Progress 12.4

1. In which direction along the template DNA strand does transcription proceed, and in which direction is the mRNA molecule built?
2. What are the three major modifications that occur during the processing of an mRNA?

12.5 Second Step: Translation

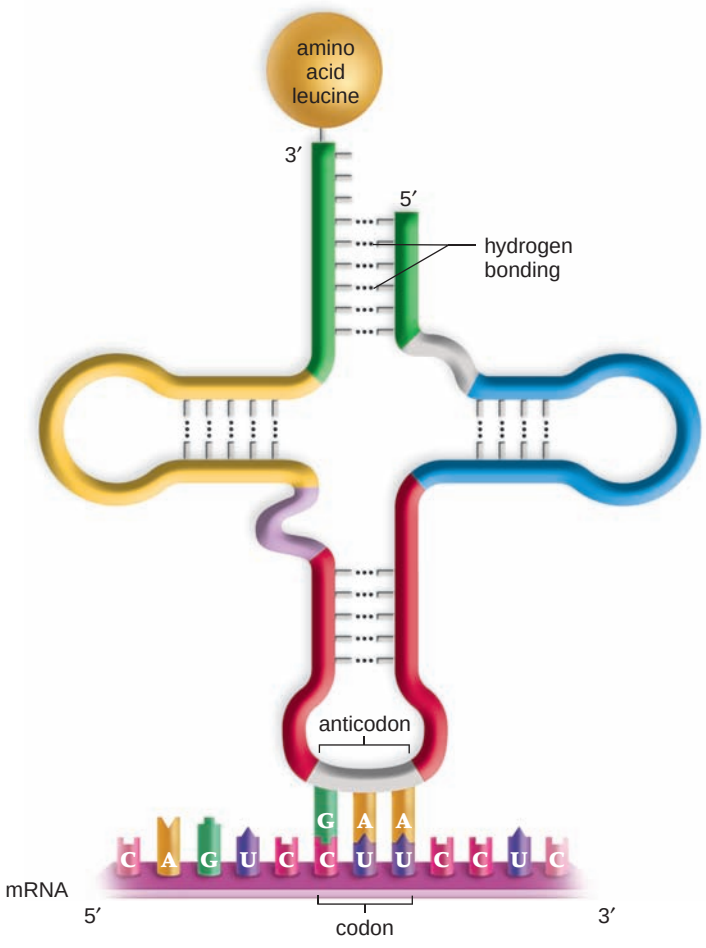
Translation, which takes place in the cytoplasm of eukaryotic cells, is the second step by which gene expression leads to protein synthesis. During translation, the sequence of codons in the mRNA at a ribosome directs the sequence of amino acids in a polypeptide. In other words, one language (nucleic acids) gets translated into another language (protein).

The Role of Transfer RNA

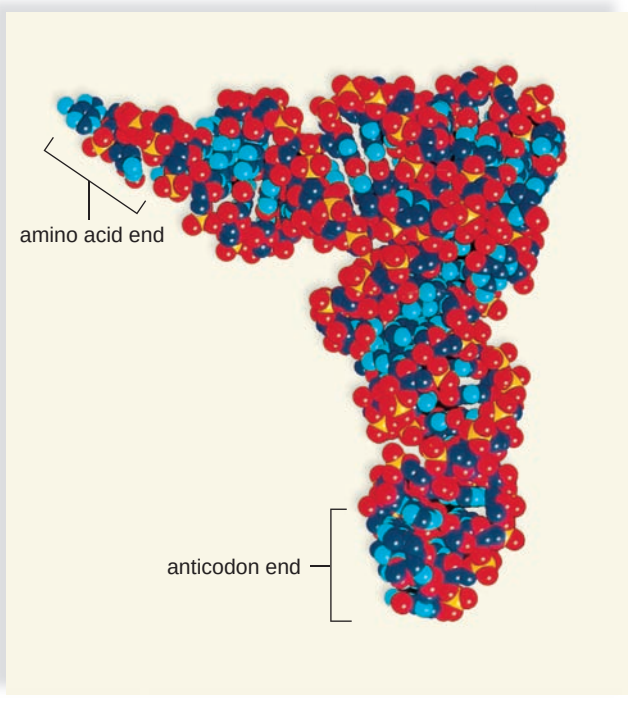
Transfer RNA (tRNA) molecules transfer amino acids to the ribosomes. A tRNA molecule is a single-stranded nucleic acid that doubles back on itself to create regions where complementary bases are hydrogen-bonded to one another. The structure of a tRNA molecule is generally drawn as a flat cloverleaf, but a space-filling model shows the molecule's three-dimensional shape (Fig. 12.14).

There is at least one tRNA molecule for each of the 20 amino acids found in proteins. The amino acid binds to the 3′ end. The opposite end of the molecule contains an **anticodon,** a group of three bases that is complementary to a specific mRNA codon. The codon and anticodon pair in an antiparallel fashion, just as two DNA strands do. For example, a tRNA that has the anticodon 5′ AAG 3′ binds to the mRNA codon 5′ CUU 3′ and carries the amino acid leucine (Fig. 12.14*a*). In the genetic code, there are 61 codons that encode for amino acids; the other three serve as stop sequences. Approximately 40 different tRNA molecules are found in most cells. There are fewer tRNAs than codons because some tRNAs can pair with more than one codon. In 1966, Francis Crick observed this phenomenon and called it the **wobble hypothesis.** He stated that the first two positions in a tRNA anticodon pair obey the A–U/G–C configuration. However, the third position can be variable. Some tRNA molecules can recognize as many as four separate codons differing only in the third nucleotide. The wobble effect helps ensure that despite changes in DNA base sequences, the correct sequence of amino acids will result in a protein.

How does the correct amino acid become attached to the correct tRNA molecule? This task is carried out by amino acid–activating enzymes, called aminoacyl-tRNA synthetases. Just as a key fits a lock, each enzyme has a recognition site for the amino acid to be joined to a particular tRNA. This is an energy-requiring process that uses ATP. Once the amino acid–tRNA complex is formed, it travels through the cytoplasm to a ribosome, where protein synthesis is occurring.



a.



b.

FIGURE 12.14 Structure of a transfer RNA (tRNA) molecule.

a. Complementary base pairing indicated by hydrogen bonding occurs between nucleotides of the molecule, and this causes it to form its characteristic loops. The anticodon that base-pairs with a particular messenger RNA (mRNA) codon occurs at one end of the folded molecule; the other two loops help hold the molecule at the ribosome. An appropriate amino acid is attached at the 3′ end of the molecule. For this mRNA codon and tRNA anticodon, the specific amino acid is leucine. **b.** Space-filling model of tRNA molecule.

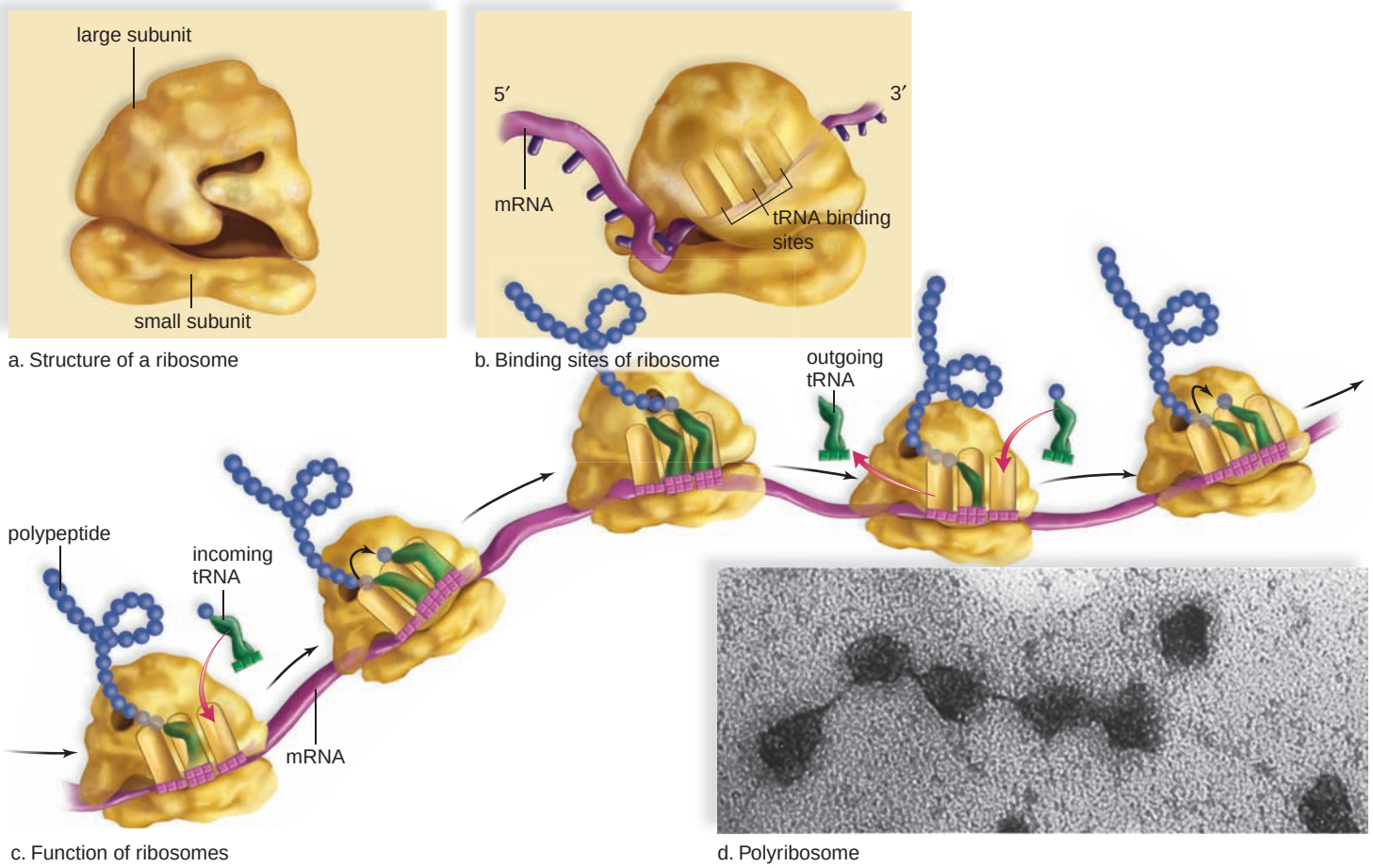


FIGURE 12.15 Ribosome structure and function.

a. Side view of a ribosome shows that it is composed of two subunits: a small subunit and a large subunit. **b.** Frontal view of a ribosome shows its binding sites. mRNA is bound to the small subunit, and the large subunit has three binding sites for tRNAs. **c.** Overview of protein synthesis. A polypeptide increases by one amino acid at a time because a peptide-bearing tRNA passes the peptide to an amino acid-bearing tRNA at a ribosome. Freed of its burden, the "empty" tRNA exits, and the peptide-bearing tRNA moves over one binding site. The polypeptide is formed as this process is repeated. **d.** Electron micrograph of a polyribosome, a number of ribosomes all translating the same mRNA molecule.

The Role of Ribosomal RNA

The structure of a ribosome is suitable to its function.

Structure of a Ribosome

In eukaryotes, ribosomal RNA (rRNA) is produced from a DNA template in the nucleolus of a nucleus. The rRNA is packaged with a variety of proteins into two ribosomal subunits, one of which is larger than the other. Then the subunits move separately through nuclear envelope pores into the cytoplasm, where they combine when translation begins (Fig. 12.15*a*). Ribosomes can remain in the cytoplasm, or they can become attached to endoplasmic reticulum.

Function of a Ribosome

Both prokaryotic and eukaryotic cells contain thousands of ribosomes per cell because they play a significant role in protein synthesis. Ribosomes have a binding site for mRNA and three binding sites for transfer RNA (tRNA) molecules (Fig. 12.15*b*). The tRNA binding sites facilitate complementary base pairing between tRNA anticodons and mRNA codons. A ribosomal RNA (i.e., a ribozyme) is now known to join one amino acid to another amino acid as a polypeptide is synthesized by the ribosome.

When a ribosome moves down an mRNA molecule, the polypeptide increases by one amino acid at a time (Fig. 12.15*c*). Translation terminates at a stop codon. Once transcription is complete, the polypeptide dissociates from the translation complex and adopts its normal shape. In Chapter 3 we observed that a polypeptide twists and bends into a definite shape. This so-called folding process begins as soon as the polypeptide emerges from a ribosome, and chaperone molecules are often present in the cytoplasm and in the ER to make sure that all goes well. Some proteins contain more than one polypeptide, and if so they join to produce the final three-dimensional structure of a functional protein.

Several ribosomes are often attached to and translating the same mRNA. As soon as the initial portion of mRNA has been translated by one ribosome, and the ribosome has begun to move down the mRNA, another ribosome attaches to the mRNA. The entire complex is called a **polyribosome** (Fig. 12.15*d*) and greatly increases the efficiency of translation.

Translation Requires Three Steps

During translation, the codons of an mRNA base pair with the anticodons of tRNA molecules carrying specific amino acids. The order of the codons determines the order of the tRNA molecules at a ribosome and the sequence of amino acids in a polypeptide. The process of translation must be extremely orderly so that the amino acids of a polypeptide are sequenced correctly.

Protein synthesis involves three steps: initiation, elongation, and termination. Enzymes are required for each of the three steps to function properly. The first two steps, initiation and elongation, require energy.

Initiation

Initiation is the step that brings all the translation components together. Proteins called initiation factors are required to assemble the small ribosomal subunit, mRNA, initiator tRNA, and the large ribosomal subunit for the start of protein synthesis.

Initiation is shown in Figure 12.16. In prokaryotes, a small ribosomal subunit attaches to the mRNA in the vicinity of the *start codon* (AUG). The first or initiator tRNA pairs with this codon. Then, a large ribosomal subunit joins to the small subunit (Fig. 12.16). Although similar in many ways, initiation in eukaryotes is much more complex and complicated.

As already discussed, a ribosome has three binding sites for tRNAs. One of these is called the E (for exit) site, second is the P (for peptide) site, and the third is the A (for amino acid) site. The initiator tRNA happens to be capable of binding to the P site, even though it carries only the amino acid methionine (see Fig. 12.10). The A site is for tRNA carrying the next amino acid, and the E site is for any tRNAs that are leaving a ribosome. Following initiation, translation continues with elongation and then termination.

Elongation

Elongation is the protein synthesis step in which a polypeptide increases in length one amino acid at a time. In addition to the participation of tRNAs, elongation requires elongation factors, which facilitate the binding of tRNA anticodons to mRNA codons at a ribosome.

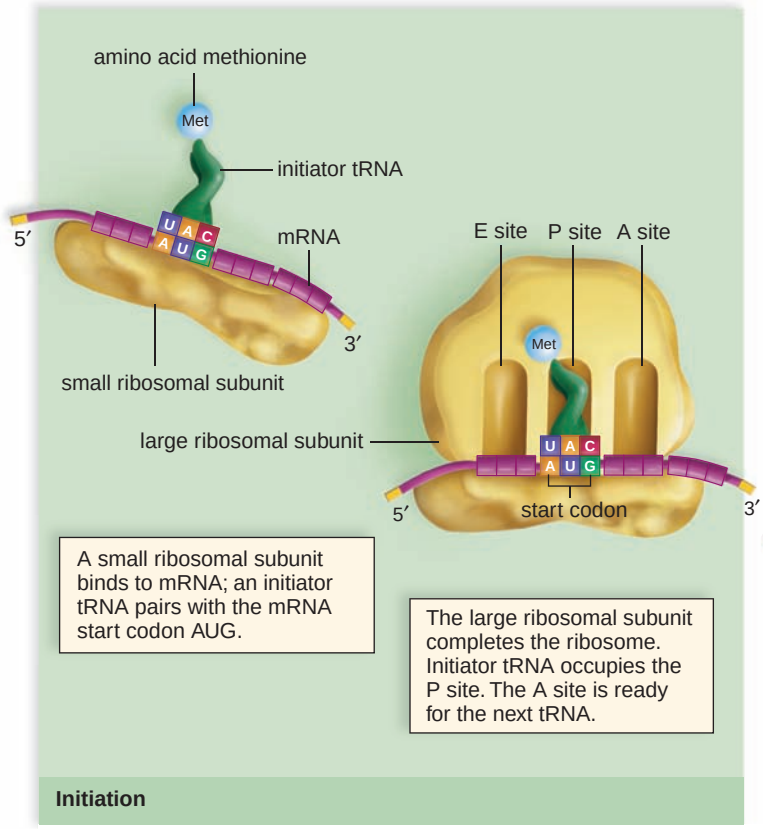


FIGURE 12.16 Initiation.

In prokaryotes, participants in the translation process assemble as shown. The first amino acid is typically methionine.

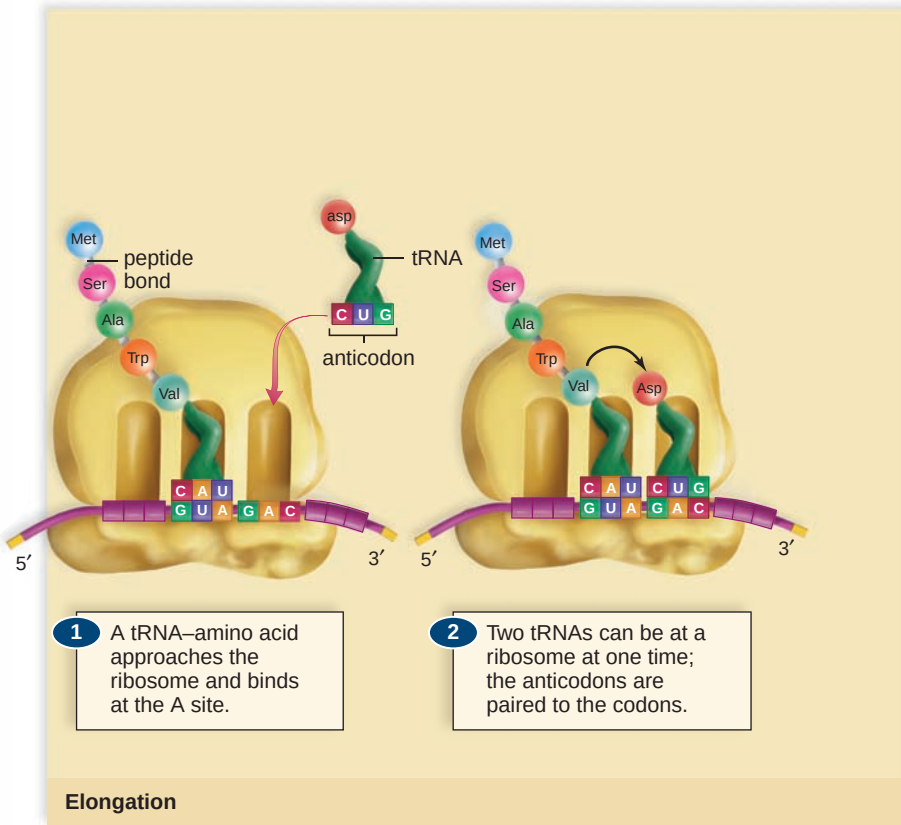


FIGURE 12.17 Elongation.

Note that a polypeptide is already at the P site. During elongation, polypeptide synthesis occurs as amino acids are added one at a time to the growing chain.

1 Elongation is shown in Figure 12.17, where a tRNA with an attached peptide is already at the P site, and a tRNA carrying its appropriate amino acid is just arriving at the A site. 2 Once a ribosome has verified that the incoming tRNA matches the codon and is firmly in place at the A site, the peptide will be transferred to this tRNA. A ribozyme, which is a part of the larger ribosomal subunit, and energy are needed to bring about this transfer. 3 Following peptide bond formation the peptide is one amino acid longer than it was before. 4 Next, **translocation** occurs: The ribosome moves forward, and the peptide-bearing tRNA is now at the P site of the ribosome. The spent tRNA is now at the E site, and it exits. A new codon is at the A site and is ready to receive another tRNA.

The complete cycle—complementary base pairing of new tRNA, transfer of peptide chain, and translocation—is repeated at a rapid rate (about 15 times each second in the bacterium *Escherichia coli*).

Eventually, the ribosome reaches a stop codon, and termination occurs, during which the polypeptide is released.

Termination

Termination is the final step in protein synthesis. During termination, as shown in Figure 12.18, the polypeptide and the assembled components that carried out protein synthesis are separated from one another.

Termination of polypeptide synthesis occurs at a *stop codon*—that is, a codon that does not code for an amino acid. Termination requires a protein called a release factor, which can bind to a stop codon and also cleave the polypeptide from the last tRNA. After this occurs, the polypeptide is set free and begins to take on its three-dimensional shape. The ribosome dissociates into its two subunits.

The next section reviews the entire process of protein synthesis (recall that a protein contains one or more polypeptides) and the role of the rough endoplasmic reticulum in the production of a polypeptide. Proteins do the work of the cell, whether they reside in a cellular membrane or free in the cytoplasm. A whole new field of biology called **proteomics** is now dedicated to understanding the structure of proteins and how they function in metabolic pathways. One of the important goals of proteomics is to understand how proteins are modified in the endoplasmic reticulum and the Golgi apparatus.

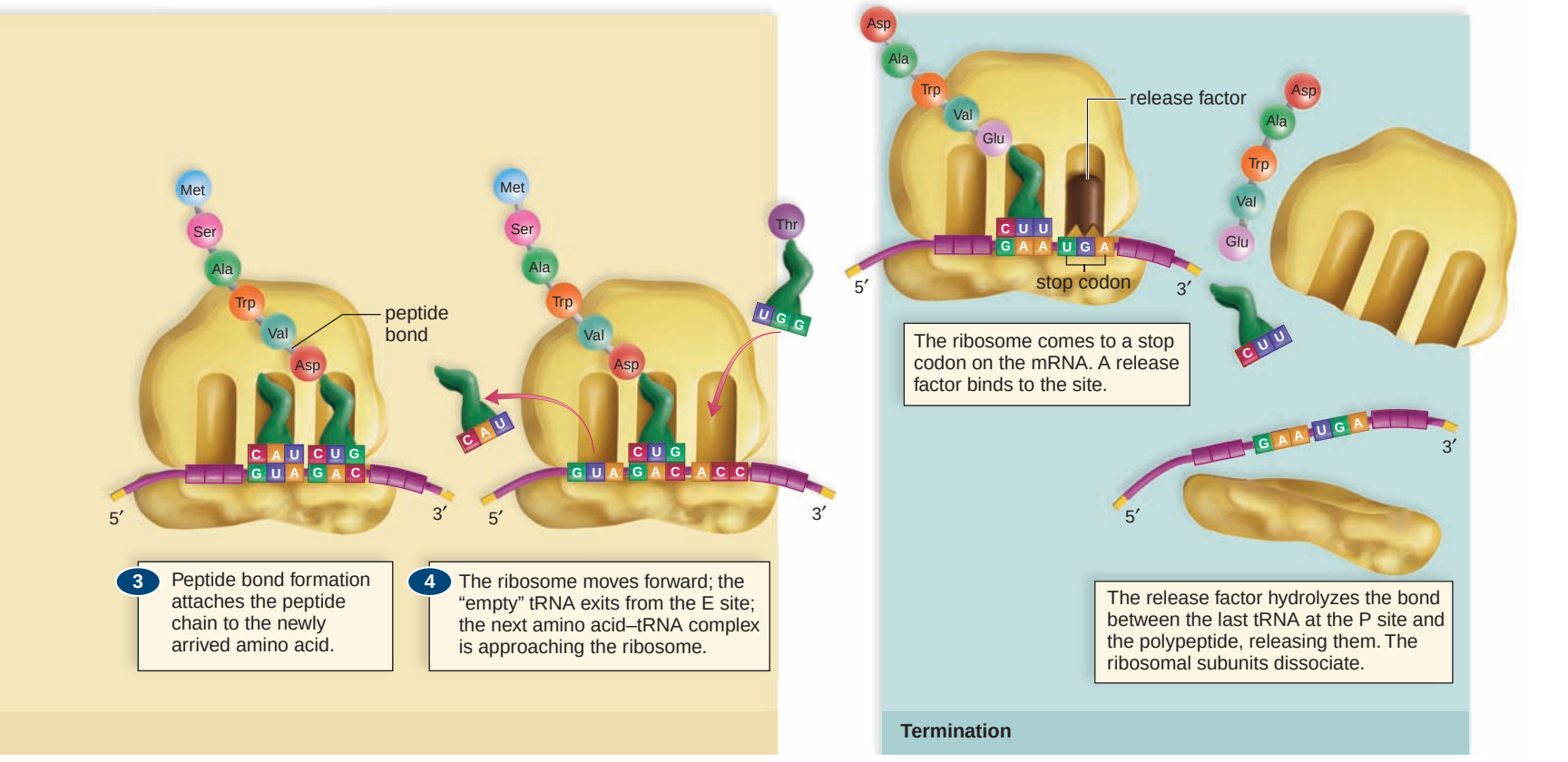


FIGURE 12.18 Termination.

During termination, the finished polypeptide is released, as is the mRNA and the last tRNA.

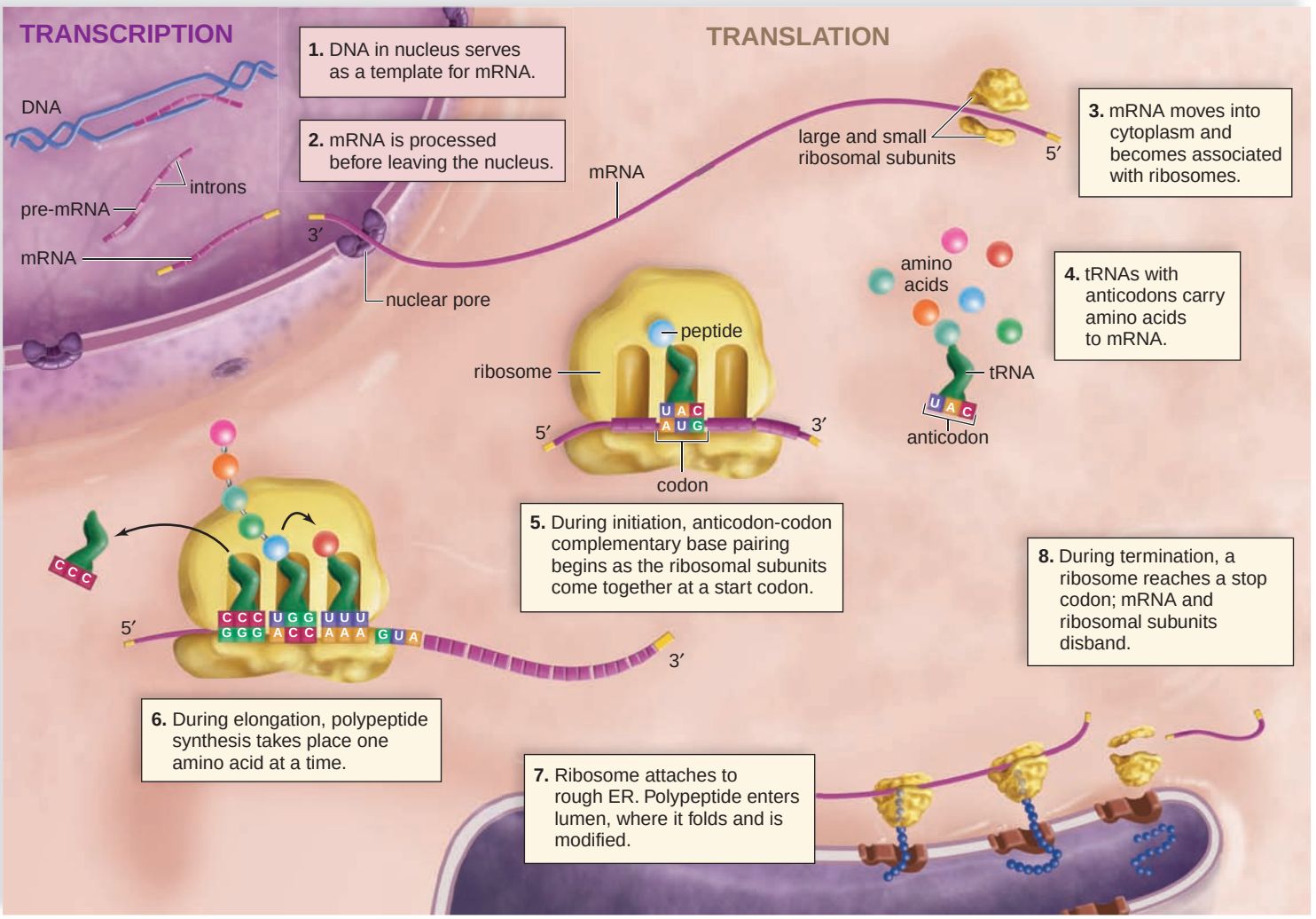


FIGURE 12.19 **Summary of protein synthesis in eukaryotes.**

Gene Expression

A gene has been expressed once its product, a protein (or an RNA), is made and is operating in the cell. For a protein, gene expression requires transcription and translation (Fig. 12.19) and it also requires that the protein be active as discussed in the next chapter.

Translation occurs at ribosomes. Some ribosomes (polyribosomes) remain free in the cytoplasm, and some become attached to rough ER. The first few amino acids of a polypeptide act as a signal peptide that indicates where the polypeptide belongs in the cell or if it is to be secreted from the cell. Polypeptides that are to be secreted enter the lumen of the ER by way of a channel, and are then folded and further processed by the addition of sugars, phosphates, or lipids. Transport vesicles carry the proteins between organelles and to the plasma membrane as appropriate for that protein.

Check Your Progress 12.5

1. What is the role of transfer RNA in translation?
2. Briefly describe the structure of a ribosome.
3. Describe the three major steps of translation.

12.6 Structure of the Eukaryotic Chromosome

Only in recent years have investigators been able to produce models suggesting how chromosomes are organized. A eukaryotic chromosome contains a single double helix DNA molecule, but is composed of more than 50% protein. Some of these proteins are concerned with DNA and RNA synthesis, but a large majority, termed **histones,** play primarily a structural role. The five primary types of histone molecules are designated H1, H2A, H2B, H3, and H4 (see Fig. 13.5*b*). Remarkably, the amino acid sequences of H3 and H4 vary little between organisms. For example, the H4 of peas is only two amino acids different from the H4 of cattle. This similarity suggests that few mutations in the histone proteins have occurred during the course of evolution and that the histones, therefore, have important functions.

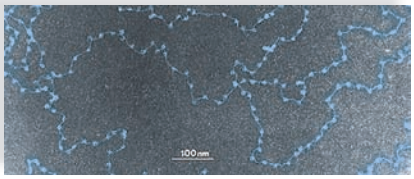
A human cell contains at least 2 m of DNA. Yet, all of this DNA is packed into a nucleus that is about 5 μm in diameter. The histones are responsible for packaging the DNA so that it can fit into such a small space. First, the DNA double helix is wound at intervals around a core of eight

histone molecules (two copies each of H2A, H2B, H3, and H4), giving the appearance of a string of beads (Fig. 12.20*a*). Each bead is called a **nucleosome,** and the nucleosomes are said to be joined by "linker" DNA. This string is compacted by folding into a zigzag structure, further shortening the DNA strand (Fig. 12.20*b*). Histone H1 appears to mediate this coiling process. The fiber then loops back and forth into radial loops (Fig. 12.20*c*). This loosely coiled **euchromatin** represents the active chromatin containing genes that are being transcribed. The DNA of euchromatin may be accessed by RNA polymerase and other factors that are needed to promote transcription.

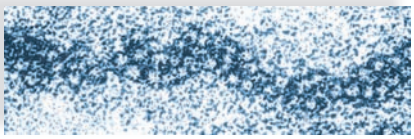
Under a microscope, one often observes dark-stained fibers within the nucleus of the cell. These areas within the nucleus represent a more highly compacted form of the chromosome called **heterochromatin** (Fig. 12.20*d*). Most chromosomes exhibit both levels of compaction in a living cell, depending on which portions of the chromosome are being used more frequently. Heterochromatin is considered inactive chromatin because the genes contained on it are infrequently transcribed, if at all. Prior to cell division, a protein scaffold helps to further condense the chromosome into a form that is characteristic of metaphase chromosomes (Fig. 12.20*e*). No doubt, compact chromosomes are easier to move about than extended chromatin.

Check Your Progress 12.6

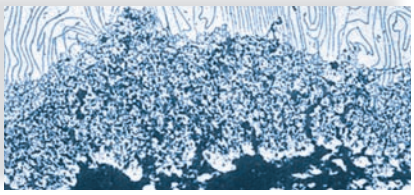
1. What is the typical compaction state of euchromatin, and how does this differ from heterochromatin?



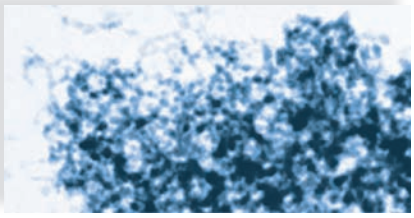
a. Nucleosomes ("beads on a string")



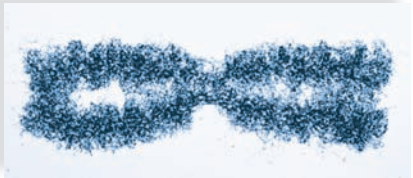
b. 30-nm fiber



c. Radial loop domains



d. Heterochromatin



e. Metaphase chromosome

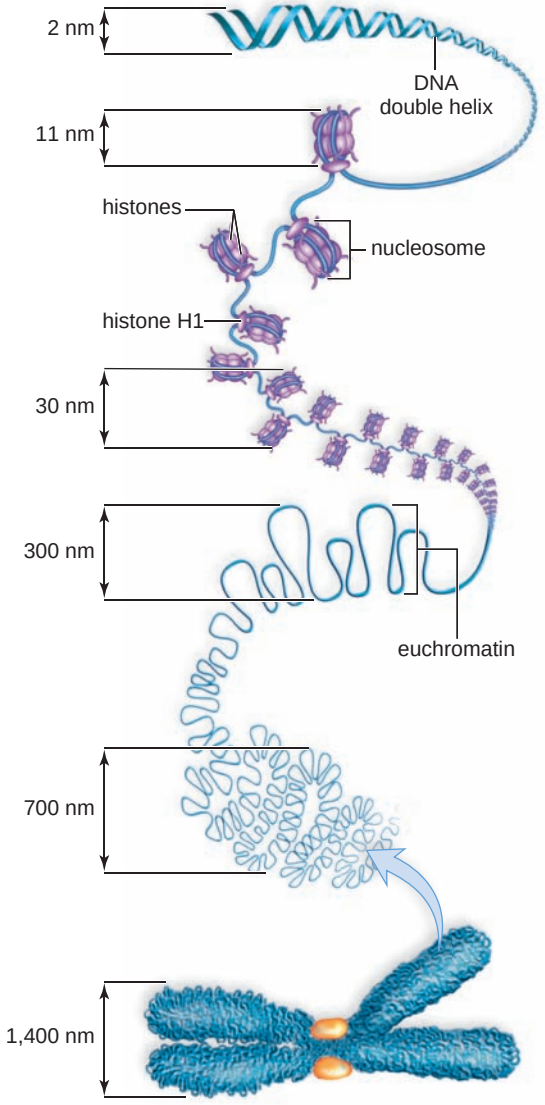


FIGURE 12.20 Structure of eukaryotic chromosomes.

The DNA molecule of a chromosome is compacted at several levels. **a.** The DNA strand is wound around histones to form nucleosomes. **b.** The strand is further shortened by folding it into a zigzag structure. **c.** The fiber loops back and forth into radial loops. **d.** In heterochromatin, additional proteins further compact the radial loops. **e.** A metaphase chromosome forms.

Connecting the Concepts

Early investigators who did their work between 1950 and 1990 came to the conclusion that DNA is organized into discrete units called genes. Genes specify proteins through the steps of transcription and translation. During transcription, a strand of DNA is used as a template for the production of an mRNA molecule. The actions of RNA polymerase, the enzyme that carries out transcription, and DNA polymerase, which is required for DNA replication, are similar enough to suggest that both enzymes evolved from a common ancestral enzyme.

Scientists are now discovering that the rest of the DNA that does not specify proteins may also have valuable functions. Specifically, it now appears that RNA may play a prominent role in the regulation of the genome. Some believe this is evidence that RNA may have preceded DNA in the evolutionary history of cells. Many biologists believe that we need a new definition of a gene that recognizes that much of our DNA results in RNA molecules rather than protein products. Nevertheless, both protein-coding and non-protein-coding DNA provides the blueprint for building and developing an entire organism. But just as a blueprint is useless without a team of engineers, architects, and construction workers to execute it, the expression of genes requires a large cadre of proteins and other factors to control it. As you will see in the following chapter, regulatory proteins may turn genes on or off, and genes can be combined in many different ways to alter the proteins that are made. Together, these mechanisms contribute to the great complexity and diversity of living organisms.

summary

12.1 The Genetic Material

Early work illustrated that DNA was the hereditary material. Griffith injected strains of pneumococcus into mice and observed that when heat-killed S strain bacteria were injected along with live R strain bacteria, virulent S strain bacteria were recovered from the dead mice. Griffith said that the R strain had been transformed by some substance passing from the dead S strain to the live R strain. Twenty years later, Avery and his colleagues reported that the transforming substance is DNA.

To study the structure of DNA, Chargaff performed a chemical analysis of DNA and found that A = T and G = C, and that the amount of purine equals the amount of pyrimidine. Franklin prepared an X-ray photograph of DNA that showed it is helical, has repeating structural features, and has certain dimensions. Watson and Crick built a model of DNA in which the sugar-phosphate molecules made up the sides of a twisted ladder, and the complementary-paired bases were the rungs of the ladder.

12.2 Replication of DNA

The Watson and Crick model immediately suggested a method by which DNA could be replicated. Basically, the two strands unwind and unzip, and each parental strand acts as a template for a new (daughter) strand. In the end, each new helix is like the other and like the parental helix.

The enzyme DNA polymerase joins the nucleotides together and proofreads them to make sure the bases have been paired correctly. Incorrect base pairs that survive the process are a mutation. Replication in prokaryotes typically proceeds in both directions from one point of origin to a termination region until there are two copies of the circular chromosome. Replication in eukaryotes has many points of origin and many bubbles (places where the DNA strands are separating and replication is occurring). Replication occurs at the ends of the bubbles—at replication forks. Since eukaryotes have linear chromosomes, they cannot replicate the very ends of them. Therefore, the ends (telomeres) get shorter with each replication.

12.3 The Genetic Code of Life

The central dogma of molecular biology says that (1) DNA is a template for its own replication and also for RNA formation during transcription, and (2) the sequence of nucleotides in mRNA directs the correct sequence of amino acids of a polypeptide during translation.

The genetic code is a triplet code, and each codon (code word) consists of three bases. The code is degenerate—that is, more than one codon exists for most amino acids. There are also one start and three stop codons. The genetic code is considered universal, but there are a few exceptions.

12.4 First Step: Transcription

Transcription to form messenger RNA (mRNA) begins when RNA polymerase attaches to the promoter of a gene. Elongation occurs until RNA polymerase reaches a stop sequence. The mRNA is processed following transcription. A cap is put onto the 5′ end, and a poly-A tail is put onto the 3′ end, and introns are removed in eukaryotes by spliceosomes. Small nuclear RNAs (snRNAs) present in spliceosomes help identify the introns to be removed. Small nucleolar RNAs (snoRNAs) perform the same processing function in the nucleolus. These snRNAs play a role in alternative mRNA splicing, which allows a single eukaryotic gene to code for different proteins, depending on which segments of the gene serve as introns and which serve as exons. Some introns serve as microRNAs (mRNAs), which help regulate the translation of mRNAs. Research is now directed to discovering the many ways small RNAs influence the production of proteins in a cell.

12.5 Second Step: Translation

Translation requires mRNA, transfer RNA (tRNA), and ribosomal RNA (rRNA). Each tRNA has an anticodon at one end and an amino acid at the other; amino acid–activating enzymes ensure that the correct amino acid is attached to the correct tRNA. When tRNAs bind with their codon at a ribosome, the amino acids are correctly sequenced in a polypeptide according to the order predetermined by DNA.

In the cytoplasm, many ribosomes move along the same mRNA at a time. Collectively, these are called a polyribosome.

Translation requires these steps: During initiation, mRNA, the first (initiator) tRNA, and the two subunits of a ribosome all come together in the proper orientation at a start codon. During elongation, as the tRNA anticodons bind to their codons, the growing peptide chain is transferred by peptide bonding to the next amino acid in a polypeptide. During termination at a stop codon, the polypeptide is cleaved from the last tRNA. The ribosome now dissociates.

12.6 Structure of the Eukaryotic Chromosome

Eukaryotic cells contain nearly 2 m of DNA, yet must pack it all into a nucleus no more than 20 μm in diameter. Thus, the DNA is compacted by winding it around DNA-binding proteins called histones to make nucleosomes. The nucleosomes are further compacted into

a zigzag structure, which is then folded upon itself many times to form radial loops, which is the usual compaction state of euchromatin. Heterochromatin is further compacted by scaffold proteins, and further compaction can be achieved prior to mitosis and meiosis.

understanding the terms

adenine (A) 214
anticodon 224
codon 221
complementary base pairing 216
cytosine (C) 214
DNA polymerase 217
DNA replication 217
double helix 215
elongation 226
euchromatin 229
exon 223
genetic code 221
guanine (G) 214
heterochromatin 229
histone 228
initiation 226
intron 223
messenger RNA (mRNA) 220
mRNA transcript 222
nucleosome 229
nucleotide 212
polyribosome 225
promoter 222
proteomics 227
replication fork 219
ribosomal RNA (rRNA) 220
ribozyme 223
RNA polymerase 222
semiconservative replication 217
telomere 218
template 217
termination 227
thymine (T) 214
transcription 220
transfer RNA (tRNA) 220
translation 220
translocation 227
triplet code 221
uracil (U) 220
wobble hypothesis 224

Match the terms to these definitions:

a. _____ Noncoding segment of DNA that is transcribed but is removed from the transcript before leaving the nucleus.
b. _____ During replication, an enzyme that joins the nucleotides complementary to a DNA template.
c. _____ A type of repetitive DNA element that may be distributed across multiple chromosomes.
d. _____ Events by which the sequence of codons in mRNA determines the sequence of amino acids in a polypeptide.

reviewing this chapter

1. List and discuss the requirements for genetic material. 212
2. How did Avery and his colleagues demonstrate that the transforming substance is DNA? 212–13
3. Describe the Watson and Crick model of DNA structure. How did it fit the data provided by Chargaff and the X-ray diffraction patterns of Franklin? 214–16
4. Explain how DNA replicates semiconservatively. What role does DNA polymerase play? What role does helicase play? 217
5. List and discuss differences between prokaryotic and eukaryotic replication of DNA. 219
6. How did investigators reason that the code must be a triplet code, and in what manner was the code cracked? Why is it said that the code is degenerate, unambiguous, and almost universal? 220–21
7. What two steps are required for the expression of a gene? 222, 224
8. What specific steps occur during transcription of RNA off a DNA template? 222
9. How is messenger RNA (mRNA) processed before leaving the eukaryotic nucleus? 222–23
10. What is the role of snRNAs in the nucleus and the role of snoRNAs in the nucleolus? 223
11. Compare the functions of mRNA, transfer RNA (tRNA), and ribosomal RNA (rRNA) during protein synthesis. What are the specific events of translation? 224–27
12. What are the various levels of chromosome structure? 228–29

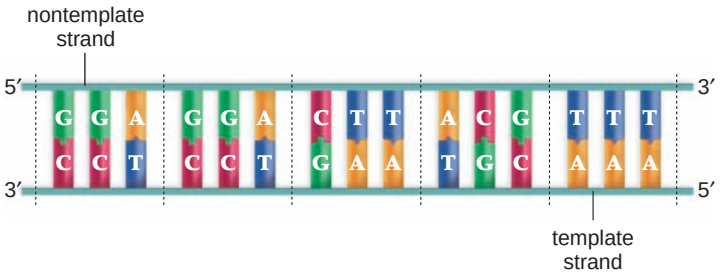
testing yourself

Choose the best answer for each question.

1. If 30% of an organism's DNA is thymine, then
   a. 70% is purine.
   b. 20% is guanine.
   c. 30% is adenine.
   d. 70% is pyrimidine.
   e. Both c and d are correct.
2. The double-helix model of DNA resembles a twisted ladder in which the rungs of the ladder are
   a. a purine paired with a pyrimidine.
   b. A paired with G and C paired with T.
   c. sugar-phosphate paired with sugar-phosphate.
   d. a 5′ end paired with a 3′ end.
   e. Both a and b are correct.
3. In a DNA molecule,
   a. the bases are covalently bonded to the sugars.
   b. the sugars are covalently bonded to the phosphates.
   c. the bases are hydrogen-bonded to one another.
   d. the nucleotides are covalently bonded to one another.
   e. All of these are correct.
4. DNA replication is said to be semiconservative because
   a. one of the new molecules conserves both of the original DNA strands.
   b. the new DNA molecule contains two new DNA strands.
   c. both of the new molecules contain one new strand and one old strand.
   d. DNA polymerase conserves both of the old strands.
5. If the sequence of bases in one strand of DNA is 5′ TAGCCT 3′, then the sequence of bases in the other strand will be
   a. 3′ TCCGAT 5′.
   b. 3′ ATCGGA 5′.
   c. 3′ TAGCCT 5′.
   d. 3′ AACGGUA 5′.
6. Transformation occurs when
   a. DNA is transformed into RNA.
   b. DNA is transformed into protein.
   c. bacteria cannot grow on penicillin.
   d. organisms receive foreign DNA and thereby acquire a new characteristic.
7. Pyrimidines
   a. are always paired with a purine.
   b. are thymine and cytosine.
   c. keep DNA from replicating too often.
   d. are adenine and guanine.
   e. Both a and b are correct.
8. Watson and Crick incorporated which of the following into their model of DNA structure?
   a. Franklin's diffraction data
   b. Chargaff's rules
   c. complementary base pairing
   d. alternating sugar-phosphate backbone
   e. All of these are correct.

9. A nucleotide
   a. is smaller than a base.
   b. is a subunit of nucleic acids.
   c. has a lot of variable parts.
   d. has at least four phosphates.
   e. always joins with other nucleotides.

10. This is a segment of a DNA molecule. What are (a) the RNA codons, (b) the tRNA anticodons, and (c) the sequence of amino acids in a protein?



11. During replication, separation of DNA strands requires
   a. backbones to split.
   b. nucleotides to join together.
   c. hydrolysis and synthesis to occur.
   d. hydrogen bonds to unzip.
   e. All of these are correct.

12. In prokaryotes,
   a. replication can occur in two directions at once because their DNA molecule is circular.
   b. bubbles thereby created spread out until they meet.
   c. replication occurs at numerous replication forks.
   d. a new round of DNA replication cannot begin before the previous round is complete.
   e. Both a and b are correct.

13. The central dogma of molecular biology
   a. states that DNA is a template for all RNA production.
   b. states that DNA is a template only for DNA replication.
   c. states that translation precedes transcription.
   d. states that RNA is a template for DNA replication.
   e. All of these are correct.

14. Transcription of a gene results in the production of
   a. an mRNA.
   b. proteins.
   c. an rRNA.
   d. ribozymes.

15. Which of these does not characterize the process of transcription? Choose more than one answer if correct.
   a. RNA is made with one strand of the DNA serving as a template.
   b. In making RNA, the base uracil of RNA pairs with the base thymine of DNA.
   c. The enzyme RNA polymerase synthesizes RNA.
   d. RNA is made in the cytoplasm of eukaryotic cells.

16. Because there are more codons than amino acids,
   a. some amino acids are specified by more than one codon.
   b. some codons specify more than one amino acid.
   c. some codons do not specify any amino acid.
   d. some amino acids do not have codons.

17. If the sequence of bases in the coding strand of a DNA is TAGC, then the sequence of bases in the mRNA will be
   a. AUCG.
   b. TAGC.
   c. UAGC.
   d. CGAU.

18. During protein synthesis, an anticodon on transfer RNA (tRNA) pairs with
   a. DNA nucleotide bases.
   b. ribosomal RNA (rRNA) nucleotide bases.
   c. messenger RNA (mRNA) nucleotide bases.
   d. other tRNA nucleotide bases.
   e. Any one of these can occur.

19. If the sequence of DNA on the template strand of a gene is AAA, the mRNA codon produced by transcription will be _____ and will specify the amino acid _____.
   a. AAA, lysine
   b. AAA, phenylalanine
   c. TTT, arginine
   d. UUU, phenylalanine
   e. TTT, lysine

20. Euchromatin
   a. is organized into radial loops.
   b. is less condensed than heterochromatin.
   c. contains nucleosomes.
   d. All of these are correct.

21. Which of the following statements about the organization of eukaryotic genes is true? The protein-coding region of a eukaryotic gene
   a. is divided into exons.
   b. is always the same in every cell.
   c. is determined in part by small nuclear RNAs that influence which segments of a gene will be introns.
   d. undergoes both transcription and translation.
   e. Both a and c are true.

22. Which of these can influence the final product of a eukaryotic protein-coding gene?
   a. The introns and exons of a gene,
   b. The snRNAs present in the spliceosome.
   c. The work of microRNAs, which are derived from introns.
   d. The nuclear envelope.
   e. All but d are correct.

thinking scientifically

1. How would you test a hypothesis that a genetic condition, such as neurofibromatosis, is due to a transposon?
2. Knowing that a plant will grow from a single cell in tissue culture, how could you transform a plant so that it glows in the dark?

Biology website

The companion website for *Biology* provides a wealth of information organized and integrated by chapter. You will find practice tests, animations, videos, and much more that will complement your learning and understanding of general biology.

http://www.mhhe.com/maderbiology10

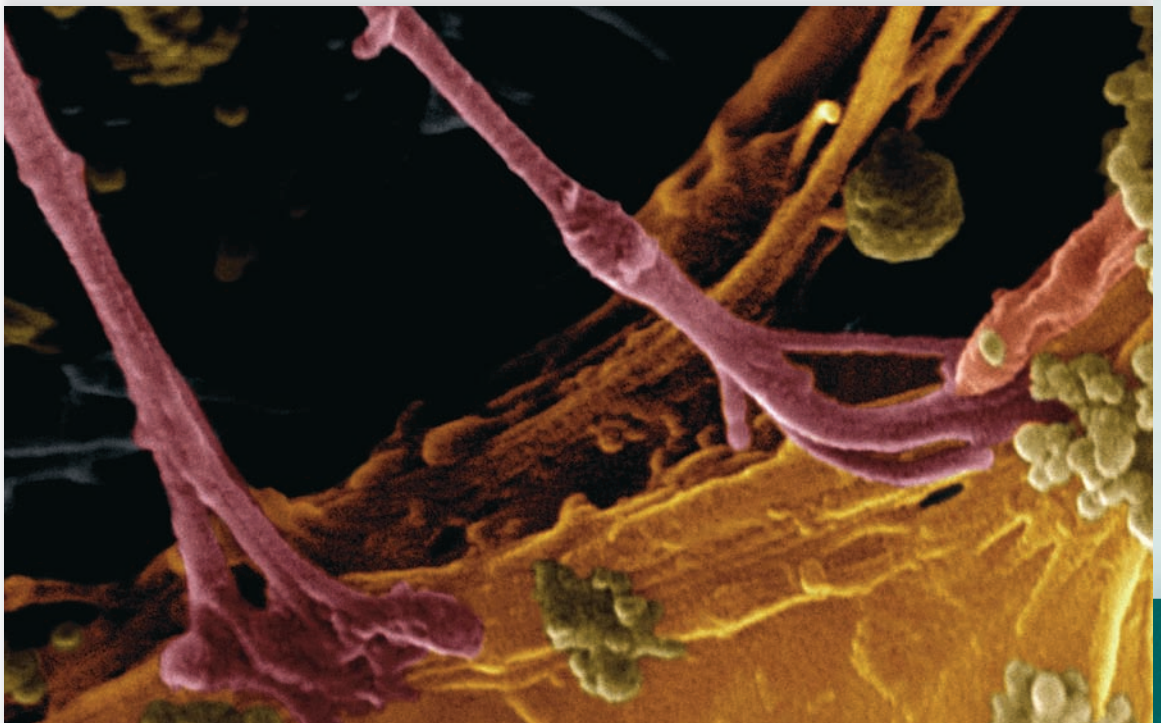
13

Regulation of Gene Activity

The human genome project revealed that humans have about 20,500 genes, scant more than a nematode, which contains nearly 20,000. So how do humans and other complex eukaryotes get by with so few genes? The answer, surprisingly, may lie in the regulation of pre-mRNA splicing to allow the production of a myriad of proteins from a single gene. DSCAM is a gene associated with Down Syndrome that is present in the brain of many animals. In fruit flies DSCAM has four regions of alternative exons. The result is over 38,000 different possible combinations of functional mRNAs and, therefore, the DSCAM gene is able to specify 38,000 different proteins. Such a huge number of proteins is sufficient to provide each nerve cell with a unique identity as it communicates with others within a brain.

Complex alternative splicing, and other regulatory mechanisms, could very well account for how humans generate so many different proteins from so few genes. This chapter introduces you to regulatory mechanisms in both prokaryotes and eukaryotes, allowing you to see how these mechanisms influence the processes of transcription and translation that you learned about in the previous chapter.

Individual neurons may use unique forms of the Dscam proteins in their plasma membranes to identify themselves.



concepts

13.1 Prokaryotic Regulation

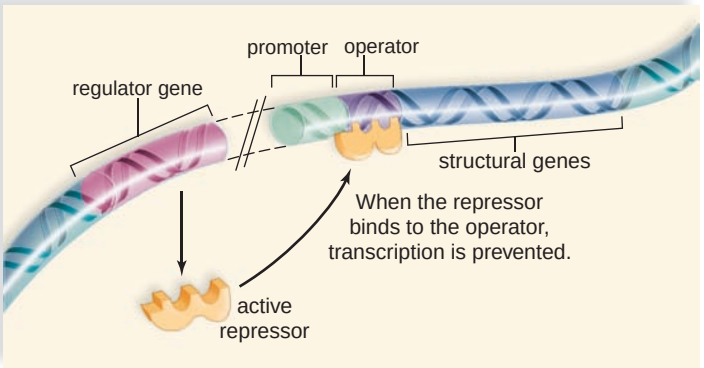
Because their environment is ever changing, bacteria do not need the same enzymes and possibly other proteins all the time. In 1961, French microbiologists François Jacob and Jacques Monod showed that *Escherichia coli* is capable of regulating the expression of its genes. They observed that the genes for a metabolic pathway, called structural genes, are grouped on a chromosome and subsequently are transcribed at the same time. Jacob and Monod, therefore, proposed the **operon** [L. *opera*, works] model to explain gene regulation in prokaryotes and later received a Nobel Prize for their investigations. An operon typically includes the following elements:

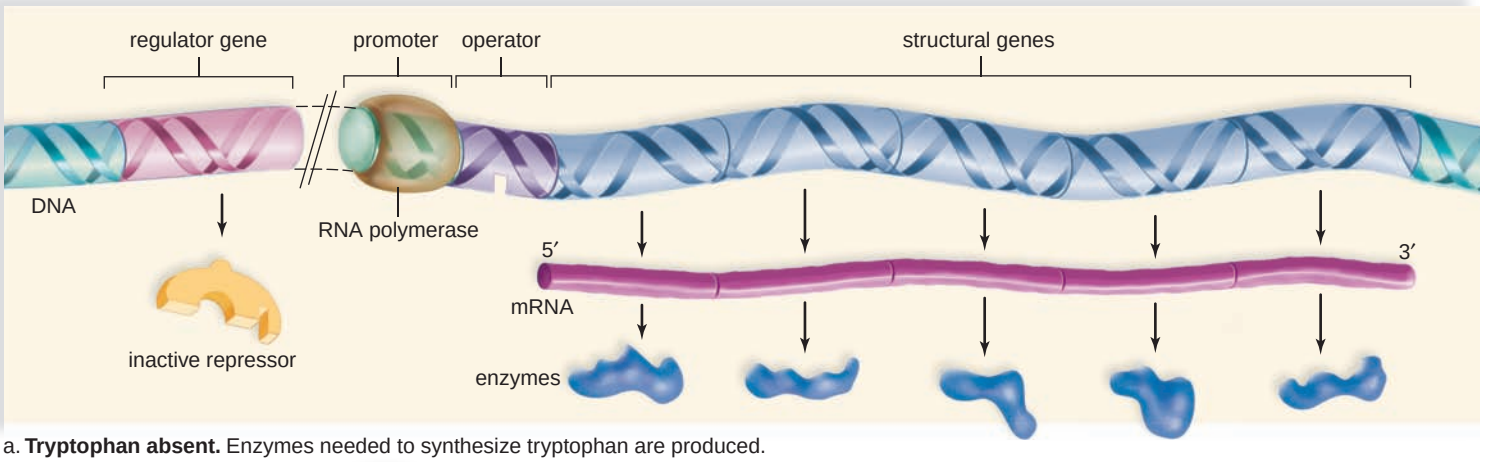
Promoter—A short sequence of DNA where RNA polymerase first attaches to begin transcription of the grouped genes. Basically, a promoter signals where transcription is to begin.

Operator—A short portion of DNA where an active repressor binds. When an active **repressor** binds to the operator, RNA polymerase cannot attach to the promoter, and transcription cannot occur. In this way, the operator controls transcription of structural genes.

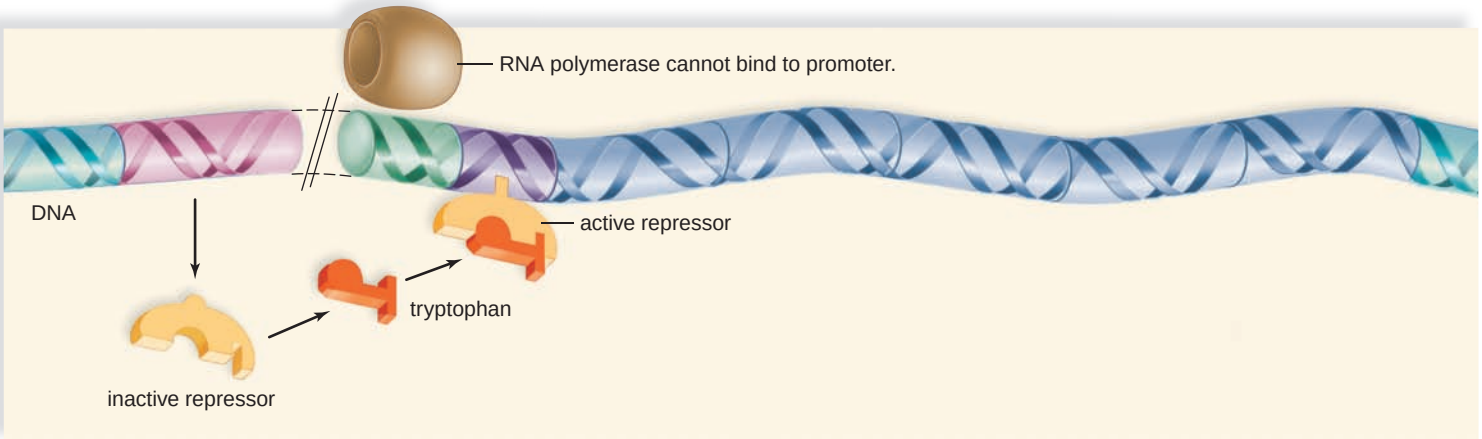
Structural genes—One to several genes coding for the primary structure of enzymes in a metabolic pathway transcribed as a unit.

A **regulator gene,** normally located outside the operon and controlled by its own promoter, codes for a repressor that controls whether the operon is active or not.





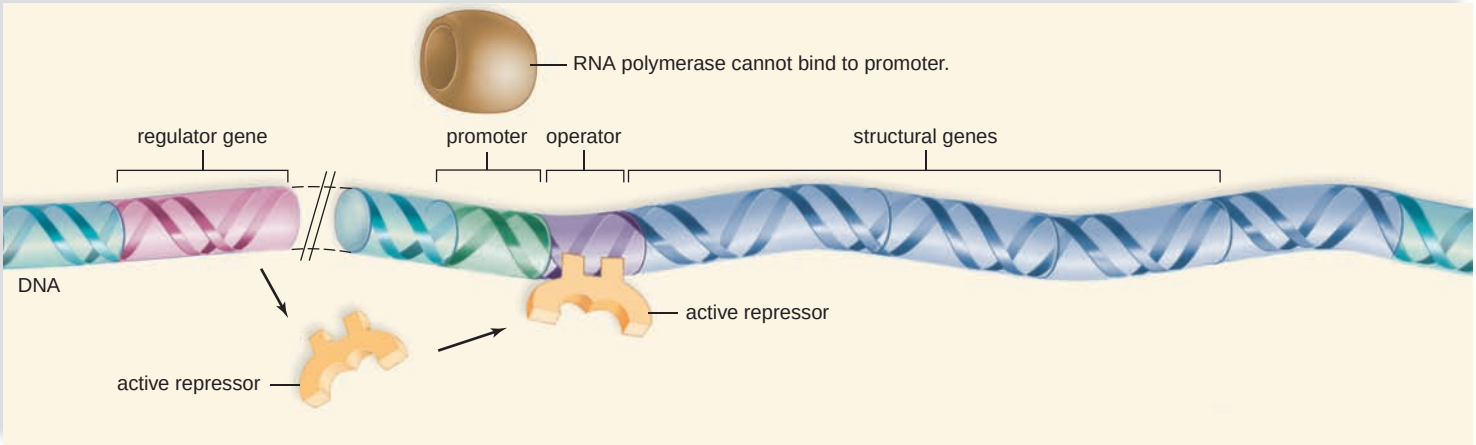
a. **Tryptophan absent.** Enzymes needed to synthesize tryptophan are produced.



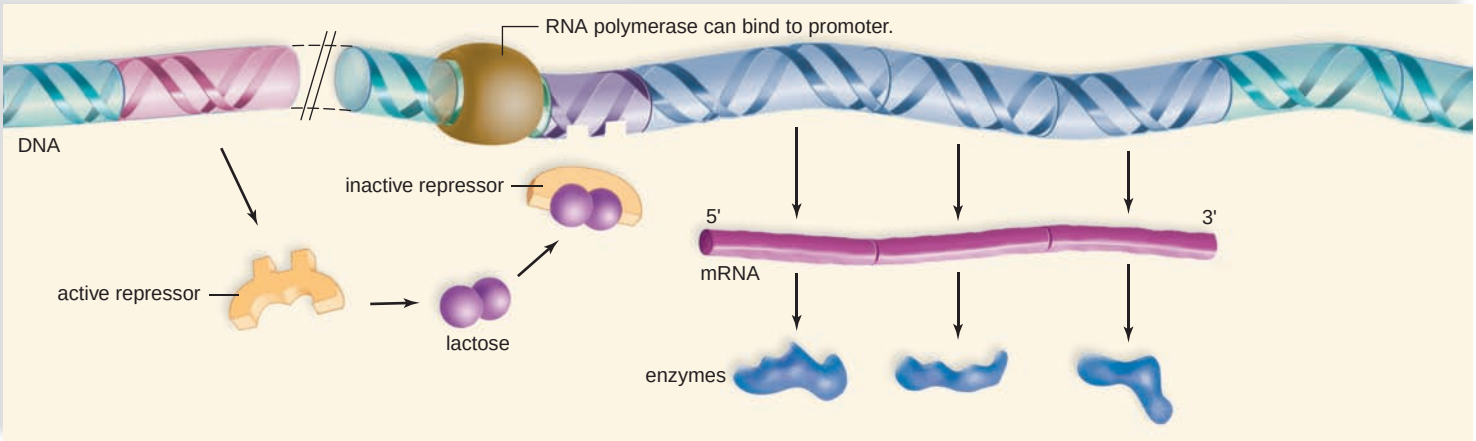
b. **Tryptophan present.** Presence of tryptophan prevents production of enzymes used to synthesize tryptophan.

FIGURE 13.1 The *trp* operon.

a. The regulator gene codes for a repressor protein that is normally inactive. RNA polymerase attaches to the promoter, and the structural genes are expressed. **b.** When the nutrient tryptophan is present, it binds to the repressor, changing its shape. Now the repressor is active and can bind to the operator. RNA polymerase cannot attach to the promoter, and the structural genes are not expressed.



a. **Lactose absent.** Enzymes needed to take up and use lactose are not produced.



b. **Lactose present.** Enzymes needed to take up and use lactose are produced only when lactose is present.

FIGURE 13.2 The *lac* operon.

a. The regulator gene codes for a repressor that is normally active. When it binds to the operator, RNA polymerase cannot attach to the promoter, and structural genes are not expressed. **b.** When lactose is present, it binds to the repressor, changing its shape so that it is inactive and cannot bind to the operator. Now, RNA polymerase binds to the promoter, and the structural genes are expressed.

The *trp* Operon

Investigators, including Jacob and Monod, found that some operons in *E. coli* usually exist in the "on" rather than "off" condition. For example, in the *trp* operon, the regulator codes for a repressor that ordinarily is unable to attach to the operator. Therefore, RNA polymerase is able to bind to the promoter, and the structural genes of the operon are ordinarily expressed (Fig. 13.1). Their products, five different enzymes, are part of an anabolic pathway for the synthesis of the amino acid tryptophan.

If tryptophan happens to be already present in the medium, these enzymes are not needed by the cell, and the operon is turned off by the following method. Tryptophan binds to the repressor. A change in shape now allows the repressor to bind to the operator, and the structural genes are not expressed. The enzymes are said to be repressible, and the entire unit is called a **repressible operon.** Tryptophan is called the **corepressor.** Repressible operons are usually involved in anabolic pathways that synthesize a substance needed by the cell.

The *lac* Operon

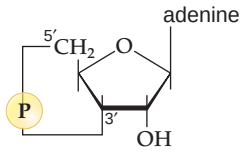
Bacteria metabolism is remarkably efficient; when there is no need for certain proteins or enzymes, the genes that are used to make them are usually inactive. For example, if the milk sugar lactose is not present, there is no need to express genes for enzymes involved in lactose catabolism. But when *E. coli* is denied glucose and is given the milk sugar lactose instead, it immediately begins to make the three enzymes needed for the metabolism of lactose. These enzymes are encoded by three genes (Fig. 13.2): One gene is for an enzyme called β-galactosidase, which breaks down the disaccharide lactose to glucose and galactose; a second gene codes for a permease that facilitates the entry of lactose into the cell; and a third gene codes for an enzyme called transacetylase, which has an accessory function in lactose metabolism.

The three structural genes are adjacent to one another on the chromosome and are under the control of a single promoter and a single operator. The regulator gene codes for a *lac* operon repressor that ordinarily binds to the operator and prevents transcription of the three genes. But when glucose is absent and lactose (or more correctly, allolactose, an isomer formed from lactose) is present, lactose binds to the repressor, and the repressor undergoes a change in shape that prevents it from binding to the operator. Because the repressor is unable to bind to the operator, RNA polymerase is better able to bind to the promoter. After RNA polymerase carries out transcription, the three enzymes of lactose metabolism are synthesized.

Because the presence of lactose brings about expression of genes, it is called an **inducer** of the *lac* operon: The enzymes are said to be inducible enzymes, and the entire unit is called an **inducible operon.** Inducible operons are usually necessary to catabolic pathways that break down a nutrient. Why is that beneficial? Because these enzymes need only be active when the nutrient is present.

Further Control of the lac *Operon*

E. coli preferentially breaks down glucose, and the bacterium has a way to ensure that the lactose operon is maximally turned on only when glucose is absent. A molecule called *cyclic AMP (cAMP)* accumulates when glucose is absent. Cyclic AMP, which is derived from ATP, has only one phosphate group, which is attached to ribose at two locations:

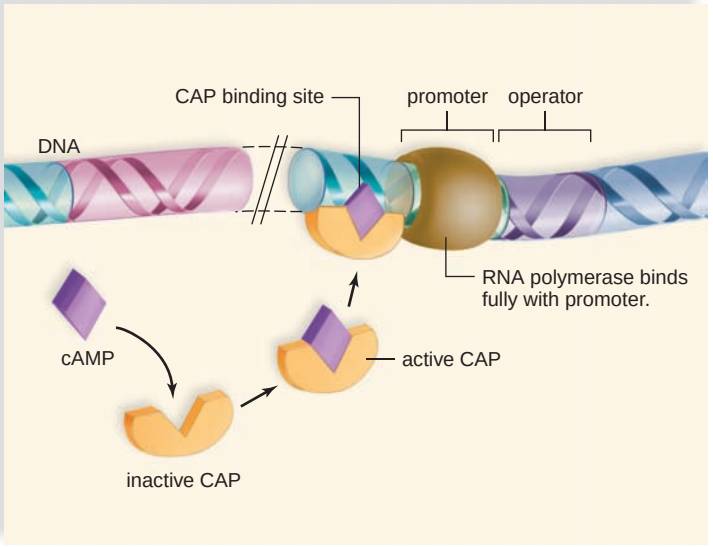


cyclic AMP
(cAMP)

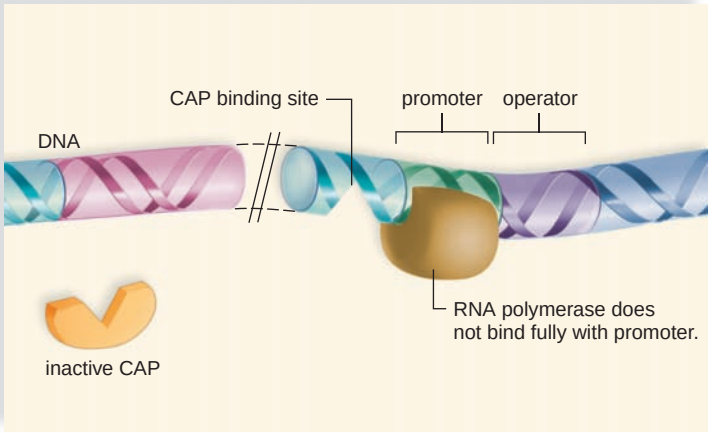
Cyclic AMP binds to a molecule called a *catabolite activator protein (CAP),* and the complex attaches to a CAP binding site next to the *lac* promoter. When CAP binds to DNA, DNA bends, exposing the promoter to RNA polymerase. RNA polymerase is now better able to bind to the promoter so that the *lac* operon structural genes are transcribed, leading to their expression (Fig. 13.3).

When glucose is present, there is little cAMP in the cell; CAP is inactive, and the lactose operon does not function maximally. CAP affects other operons as well and takes its name for activating the catabolism of various other metabolites when glucose is absent. A cell's ability to encourage the metabolism of lactose and other metabolites when glucose is absent provides a backup system for survival when the preferred energy source glucose is absent.

The CAP protein's regulation of the *lac* operon is an example of positive control. Why? Because when this molecule is active, it promotes the activity of an operon. The use of repressors, on the other hand, is an example of negative control because when active they shut down an operon. A positive control mechanism allows the cell to fine-tune its response. In the case of the *lac* operon, the operon is only maximally active when glucose is absent and lactose is present. If both glucose and lactose are present, the cell preferentially metabolizes glucose.



a. **Lactose present, glucose absent (cAMP level high)**



b. **Lactose present, glucose present (cAMP level low)**

FIGURE 13.3 Action of CAP.

When active CAP binds to its site on DNA, the RNA polymerase is better able to bind to the promoter so that the structural genes of the *lac* operon are expressed. **a.** CAP becomes active in the presence of cAMP, a molecule that is prevalent when glucose is absent. Therefore, transcription of lactose enzymes increases, and lactose is metabolized. **b.** If glucose is present, CAP is inactive, and RNA polymerase does not completely bind to the promoter. Therefore, transcription of lactose enzymes decreases, and less metabolism of lactose occurs.

Check Your Progress 13.1

1. What is an operon? Why is it advantageous for prokaryotes to organize their genes in this manner?
2. Explain the difference between positive control and negative control of gene expression.

13.2 Eukaryotic Regulation

Each cell in multicellular eukaryotes, including humans, has a copy of all genes; however, different genes are actively expressed in different cells. Muscle cells, for example, have a different set of genes that are turned on in the nucleus and a different set of proteins that are active in the cytoplasm than do nerve cells.

Like prokaryotic cells, a variety of mechanisms regulate gene expression in eukaryotic cells. These mechanisms can be grouped under five primary levels of control; three of them pertain to the nucleus, and two pertain to the cytoplasm (Fig. 13.4). In other words, control of gene activity in eukaryotes extends from transcription to protein activity. These are the types of control in eukaryotic cells that can modify the amount of the gene product:

1. *Chromatin structure:* Chromatin packing is used as a way to keep genes turned off. If genes are not accessible to RNA polymerase, they cannot be transcribed.

   In the nucleus, highly condensed chromatin is not available for transcription, while more loosely condensed chromatin is available for transcription. Chromatin structure is a part of **epigenetic** [Gk. *epi*, besides] **inheritance,** the transmission of genetic information outside the coding sequences of a gene.
2. *Transcriptional control:* The degree to which a gene is transcribed into mRNA determines the amount of gene product. In the nucleus, transcription factors may promote or repress transcription, the first step in gene expression.
3. *Posttranscriptional control:* Posttranscriptional control involves mRNA processing and how fast mRNA leaves the nucleus. We now know that mRNA processing differences can determine the type of protein product made by a cell.

   Also, mRNA processing differences can affect how fast mRNA leaves the nucleus and the amount of gene product within a given amount of time.
4. *Translational control:* Translational control occurs in the cytoplasm and affects when translation begins and how long it continues. Any influence that can cause the persistence of the 5′ cap and 3′ poly-A tail can affect the length of translation. Excised introns are now believed to be involved in a regulatory system that directly affects the life span of mRNA.

   Some mRNAs may need further processing before they are translated. The possibility of an RNA-based regulatory system is being investigated.
5. *Posttranslational control:* Posttranslational control, which also takes place in the cytoplasm, occurs after protein synthesis. Only a functional protein is an active gene product.

   The polypeptide product may have to undergo additional changes before it is biologically functional. Also, a functional enzyme is subject to feedback control so that it is no longer able to speed its reaction.

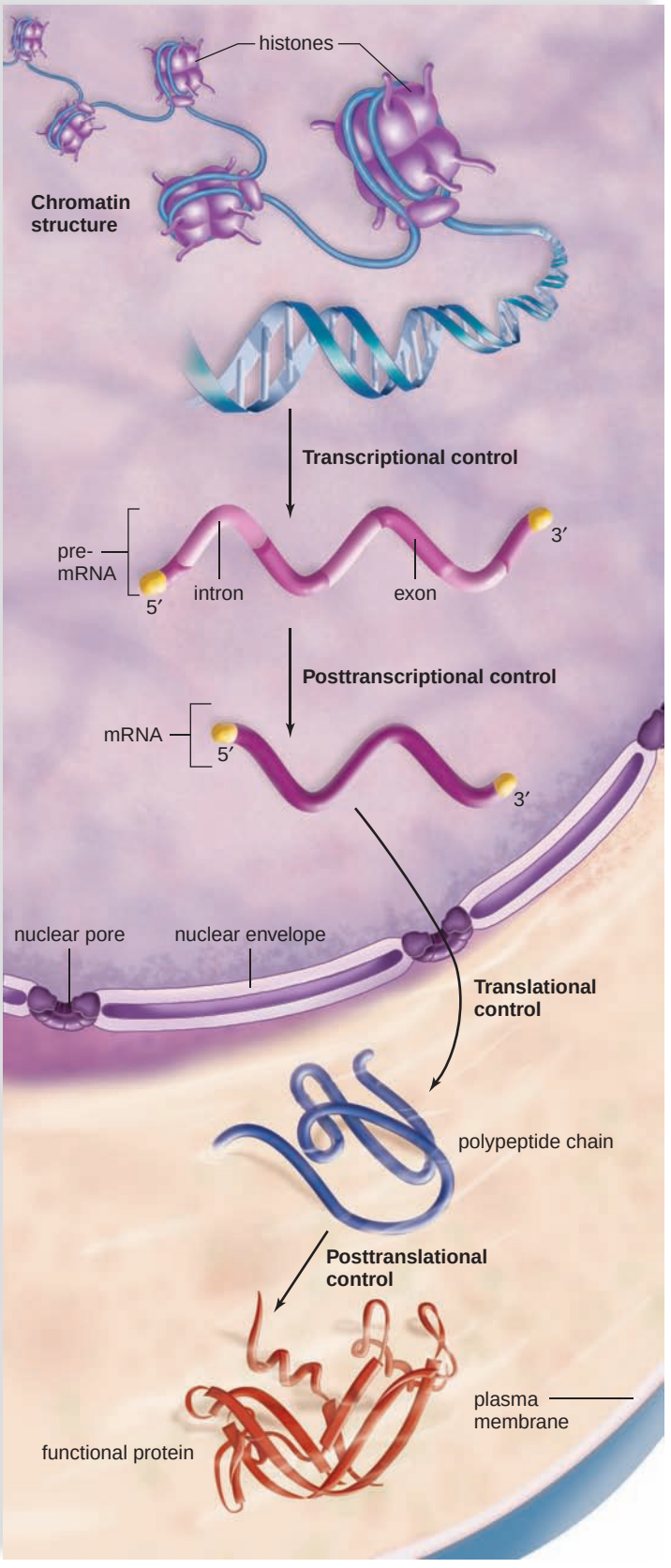


FIGURE 13.4 Levels at which control of gene expression occurs in eukaryotic cells.

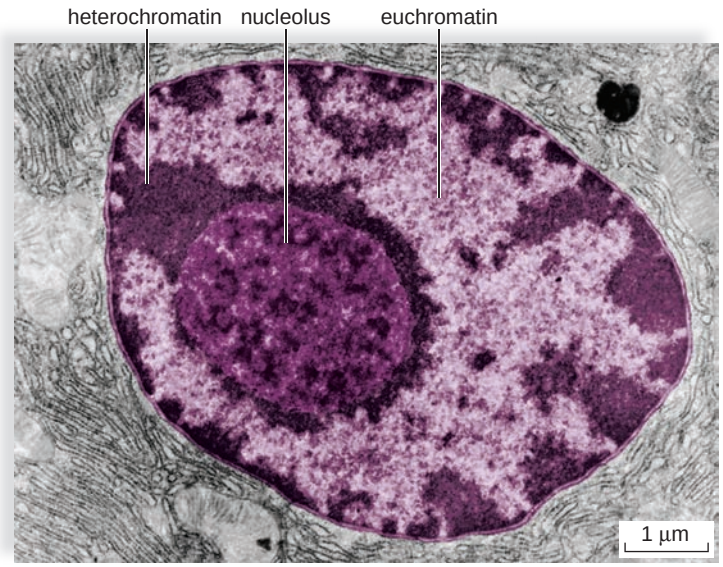
The five levels of control are (1) chromatin structure, (2) transcriptional control, and (3) posttranscriptional control, which occur in the nucleus; and (4) translational and (5) posttranslational control, which occur in the cytoplasm.

Chromatin Structure

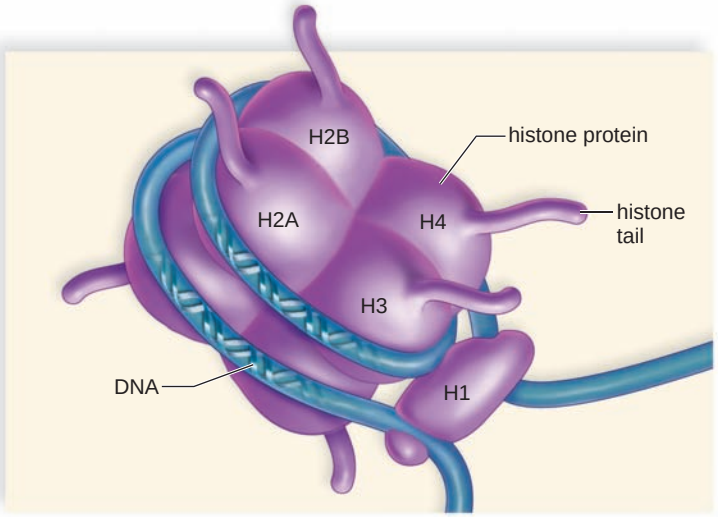
Genetic control in eukaryotes is bound to be more complicated than prokaryotes if only because they have a great deal more DNA than prokaryotes. We learned in Figure 12.20 that various levels of condensation and compaction are necessary in order to fit a very large amount of DNA into a much smaller nucleus. The degree to which chromatin is compacted greatly affects the accessibility of the chromatin to the transcriptional machinery of the cell, and thus the expression levels of the genes contained within.

In general, highly condensed heterochromatin is inaccessible to RNA polymerase, and the genes contained within are seldom or never transcribed. **Heterochromatin** appears as darkly stained portions within the nucleus in electron micrographs (Fig. 13.5*a*). A dramatic example of heterochromatin is the Barr body in mammalian females. Females have a small, darkly staining mass of condensed chromatin adhering to the inner edge of the nuclear membrane. This structure, called a **Barr body** after its discoverer, is an inactive X chromosome. One of the X chromosomes undergoes inactivation in the cells of female embryos. The inactive X chromosome does not produce gene products, and therefore female cells have a reduced amount of product from genes on the X chromosome.

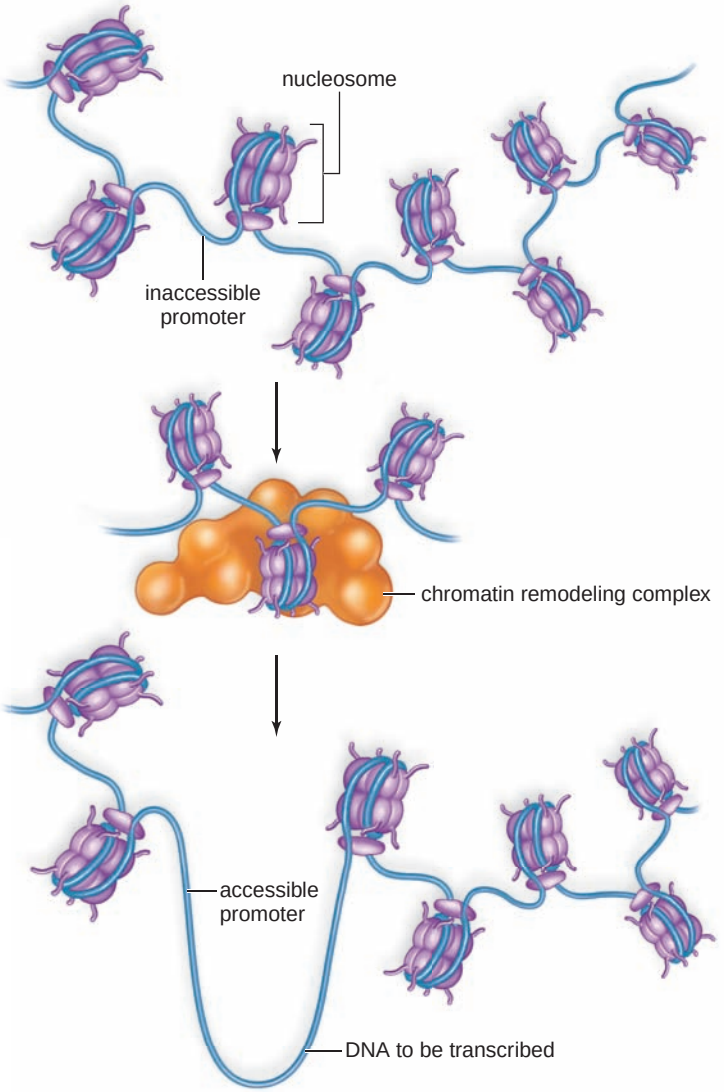
How do we know that Barr bodies are inactive X chromosomes that are not producing gene product? Suppose 50% of the cells have one X chromosome active and 50% have



a. Darkly stained heterochromatin and lightly stained euchromatin



b. A nucleosome



c. DNA unpacking

FIGURE 13.5 Chromatin structure regulates gene expression.

a. A eukaryotic nucleus contains heterochromatin (darkly stained, highly condensed chromatin) and euchromatin, which is not as condensed. **b.** Nucleosomes ordinarily prevent access to DNA so that transcription cannot take place. If histone tails are acetylated, access can be achieved; if the tails are methylated, access is more difficult. **c.** A chromatin remodeling complex works on euchromatin to make a promoter accessible for transcription.

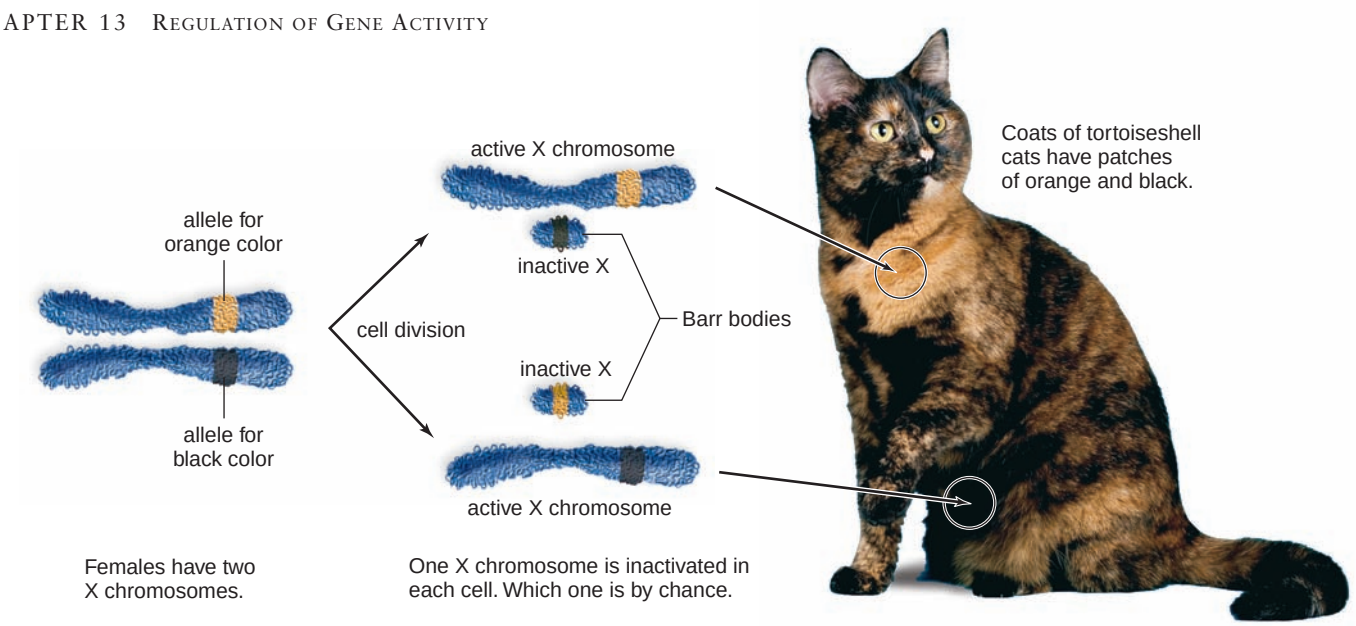


FIGURE 13.6 **X-inactivation in mammalian females.**

In cats, the alleles for black or orange coat color are carried on the X chromosomes. Random X-inactivation occurs in females. Therefore, in heterozygous females, 50% of the cells have an allele for black coat color and 50% of cells have an allele for orange coat color. The result is tortoiseshell cats that have coats with patches of both black and orange.

the other X chromosome active. Wouldn't the body of a heterozygous female be a mosaic, with "patches" of genetically different cells? This is exactly what investigators have discovered. Human females who are heterozygous for an X-linked recessive form of ocular albinism have patches of pigmented and nonpigmented cells at the back of the eye. Women heterozygous for Duchenne muscular dystrophy have patches of normal muscle tissue and degenerative muscle tissue (the normal tissue increases in size and strength to make up for the defective tissue). And women who are heterozygous for X-linked hereditary absence of sweat glands have patches of skin lacking sweat glands. The female tortoiseshell cat also provides dramatic support for a difference in X-inactivation in its cells. In these cats, an allele for black coat color is on one X chromosome, and a corresponding allele for orange coat color is on the other X chromosome. The patches of black and orange in the coat can be related to which X chromosome is in the Barr bodies of the cells found in the patches (Fig. 13.6).

DNA Unpacking

Active genes in eukaryotic cells are associated with more loosely packed chromatin called **euchromatin** (see Fig. 12.20). What regulates whether chromatin exists as heterochromatin or euchromatin? You learned in Figure 12.20 that in a 30 nm fiber a *nucleosome* is a portion of DNA wrapped around a group of histone molecules. Histone molecules have *tails,* strings of amino acids that extend beyond the main portion of a nucleosome (Fig. 13.5*b*). In heterochromatin, the histone tails tend to bear methyl groups (—CH_3); in euchromatin, the histone tails tend to be acetylated and have attached acetyl groups (—$COCH_3$).

Histones regulate accessibility to DNA, and euchromatin becomes genetically active when histones no longer bar access to DNA. When DNA in euchromatin is transcribed, a so-called *chromatin remodeling complex* pushes aside the histone portion of a nucleosome so that access to DNA is not barred and transcription can begin (Fig. 13.5*c*). After *unpacking* occurs, many decondensed loops radiate from the central axis of the chromosome. These chromosomes have been named lampbrush chromosomes because their feathery appearance resembles the brushes that were once used to clean kerosene lamps.

In addition to physically moving nucleosomes aside to expose promoters, chromatin remodeling complexes may also affect gene expression by adding acetyl or methyl groups to histone tails.

Epigenetic Inheritance

Histone modification is sometimes linked to a phenomenon termed *epigenetic inheritance,* in which the pattern of inheritance does not depend on the genes themselves. When histones are methylated, sometimes the DNA itself becomes methylated as well. Some genes undergo a phenomenon called *genomic imprinting*, and either the mother's or father's allele is methylated during gamete formation. If an inherited allele is highly methylated, the gene is not expressed, even if it is a normal gene in every other way. For traits that exhibit genomic imprinting, the expression of the gene depends on whether it was inherited from the mother or the father.

The expression epigenetic inheritance is now used broadly for other inheritance patterns that do not depend on the genes themselves. In this instance, epigenetic inheritance depends on whether acetyl and methyl groups surround and adhere to DNA. Epigenetic inheritance explains unusual inheritance patterns and also may play an important role in growth, aging, and cancer. Researchers are even hopeful that it will be easier to develop drugs to modify this level of inheritance rather than trying to change the DNA itself.

Transcriptional Control

Although eukaryotes have various levels of genetic control (see Fig. 13.4), **transcriptional control** remains the most critical of these levels. The first step toward transcription is availability of DNA for transcription, which involves DNA unpacking (see page 239). Transcriptional control also involves the participation of transcription factors, activators, and repressors.

Transcription Factors, Activators, and Repressors

Although no operons like those of prokaryotic cells have been found in eukaryotic cells, transcription is still controlled by DNA-binding proteins. Every cell contains many different types of **transcription factors,** proteins that help regulate transcription. The same transcription factors, but not the same mix, are used over again at other promoters, so it is easy to imagine that if one malfunctions, the result could be disastrous to the cell. A group of transcription factors binds to a promoter adjacent to a gene, and then the complex attracts and binds RNA polymerase, but transcription may still not begin.

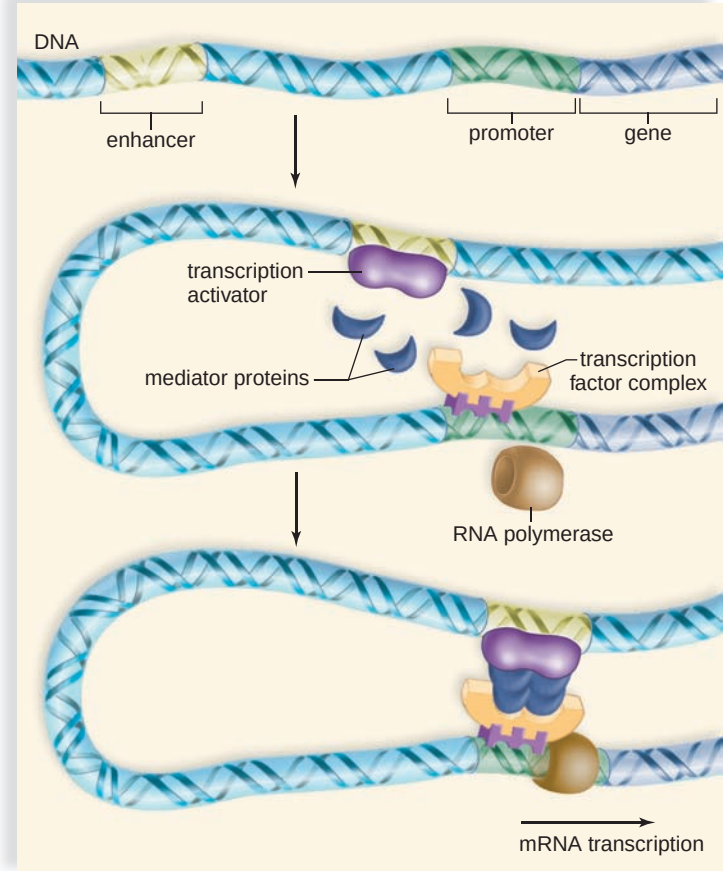


FIGURE 13.7 **Eukaryotic transcription factors.**

Transcription in eukaryotic cells requires that transcription factors bind to the promoter and transcription activators bind to an enhancer. The enhancer is far from the promoter, but the DNA loops and mediator proteins act as a bridge joining activators to factors. Only then does transcription begin.

In eukaryotes, **transcription activators** are DNA-binding proteins that speed transcription dramatically. They bind to a region of DNA called an **enhancer** that can be quite a distance from the promoter. A hairpin loop in the DNA brings the transcription activators attached to the enhancer into contact with the transcription factor complex. Likewise, the binding of repressors to silencers within the promoter may prohibit the transcription of certain genes. Most genes are subject to regulation by both activators and repressors also.

The promoter structure of eukaryotic genes is often very complex, and there is a large variety of regulatory proteins that may interact with each other and with transcription factors to affect a gene's transcription level. Mediator proteins act as a bridge between transcription factors and transcription activators at the promoter. Now RNA polymerase begins the transcription process (Fig. 13.7). Such protein-to-protein interactions are a hallmark of eukaryotic gene regulation. Together, these mechanisms can fine-tune a gene's transcription level in response to a large variety of conditions.

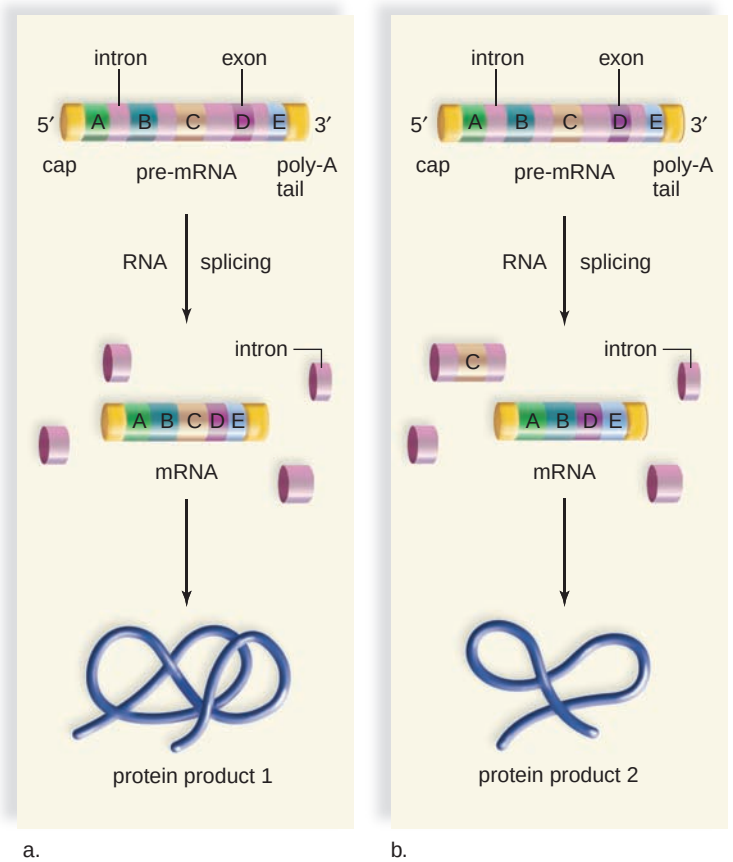
Transcription factors, activators, and repressors are always present in the nucleus of a cell, but they most likely have to be activated in some way before they will bind to DNA. Activation often occurs when they are phosphorylated by a kinase. Kinases, which add a phosphate group to molecules, and phosphatases, which remove a phosphate group, are known to be signaling proteins involved in a growth regulatory network that reaches from receptors in the plasma membrane to the genes in the nucleus.

Posttranscriptional Control

Posttranscriptional control of gene expression occurs in the nucleus and includes alternative mRNA splicing and controlling the speed with which mRNA leaves the nucleus.

During pre-mRNA splicing, introns (noncoding regions) are excised, and exons (expressed regions) are joined together to form an mRNA (see Fig. 12.13). When introns are removed from pre-mRNA, differential splicing of exons can occur, and this affects gene expression. For example, an exon that is normally included in an mRNA transcript may be skipped, and it is excised along with the flanking introns (Fig. 13.8). The resulting mature mRNA has an altered sequence, and the protein encoded differs. Sometimes introns remain in an mRNA transcript; when this occurs, the protein coding sequence will also change.

Examples of alternative pre-mRNA splicing abound. Both the hypothalamus and the thyroid gland produce a protein hormone called calcitonin, but the mRNA that leaves the nucleus is not the same in both types of cells. This results in the thyroid releasing a slightly different version of calcitonin than the hypothalamus. Evidence of alternative mRNA splicing is found in other cells, such as those that produce neurotransmitters, muscle regulatory proteins, and antibodies. This process allows humans and other complex organisms to recombine their genes in many new and novel ways to create the great variety of proteins found in these organisms. Researchers are busy determining how small nuclear



a. b.

FIGURE 13.8 Alternative processing of pre-mRNA.

Because the pre-mRNAs are processed differently in these two cells (**a** and **b**), distinct proteins result. This is a form of posttranscriptional control of gene expression.

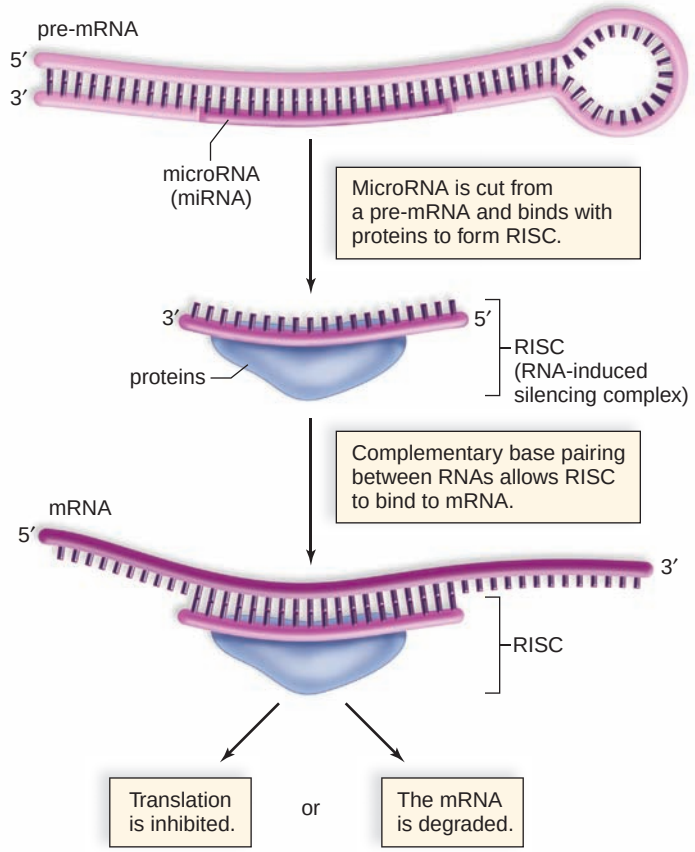


FIGURE 13.9 Function of microRNAs (miRNAs).

MicroRNAs are one of several types of RNAs now known to function in the nucleus, nucleolus, or cytoplasm to regulate protein-coding gene expression.

RNAs (snRNAs) affect the splicing of pre-mRNA. They also know that, sometimes, alternative mRNA splicing can result in the inclusion of an intron that results in destruction of the mRNA before it leaves the nucleus.

Further posttranscriptional control of gene expression is achieved by modifying the speed of transport of mRNA from the nucleus into the cytoplasm. Evidence indicates there is a difference in the length of time it takes various mRNA molecules to pass through a nuclear pore, affecting the amount of gene product realized per unit time following transcription.

Translational Control

Translational control begins when the processed mRNA molecule reaches the cytoplasm and before there is a protein product. Translational control involves the activity of mRNA for translation at the ribosome.

Presence or absence of the 5′ cap and the length of the poly-A (adenine nucleotide) tail at the 3′ end of a mature mRNA transcript can determine whether translation takes place and how long the mRNA is active. The long life of mRNAs that code for hemoglobin in mammalian red blood cells is attributed to the persistence of their 5′ end caps and their long 3′ poly-A tails. On the other hand, any influence that affects the length of the poly-A tail or leads to removal of the cap may trigger the destruction of an mRNA.

Scientists studying **microRNAs** (miRNAs) have shown that these mysterious non-protein-coding RNAs can regulate translation by causing the destruction of mRNAs before they can be translated. Cut directly from a pre-mRNA transcript, miRNAs regulate gene activity by interfering with translation of a target mRNA or RNAs (Fig 13.9). Before the miRNA leaves the nucleus, it is enzymatically processed and bound to proteins to form an RNA-induced silencing complex (RISC). An active miRNA complex is complementary to a specific target mRNA, with which it base-pairs to form a double-stranded RNA complex. The end result is inhibition of translation or, in some cases, the destruction of the mRNA itself. Much like a dimmer switch on a light, miRNAs can fine-tune the expression of genes. Scientists have since learned how to use this pathway to study the function of genes by turning them off with artificial miRNAs. Andrew Fire and Craig Mello received the 2006 Nobel Prize in Physiology or Medicine for developing this new technique.